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LIME DISINFECTION OF SEWAGE BACTERIA AT LOW TEMPERATURE

S. M. Morrison, et al

Colorado State University

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An additional effect of lime treatment of sewage is the reduction of organic and inorganic chemical loads in the effluent. The reductions at 15 C for raw and 10 C for secondary treated, measured by BOD and orthophosphate tests, reached maximum BOD removals of 77 and 94%, respectively, at pH 11 in 24 hours for raw and at pH 11.5 in 90 minutes for treated sewage. Likewise, maximum orthophosphate removals, 93 and 97%, respectively, were obtained at pH 12.0 for 60 minutes with raw and treated samples. (EPA)

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LIME DISINFECTION OF SEWAGE BACTERIA AT LOW TEMPERATURE

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ABSTRACT

Small isolated communities in cold climatic areas need a simple, inexpensive, reliable sewage system which includes disinfection. This laboratory study provides clarifying data on the action of lime as a sewage disinfectant at low temperatures. Nutrient level reductions were also studied.

Lime was added to raw and activated sludge treated sewage to attain pH intervals between 10 and 12 at temperatures of 1°, 5°, 10° and 15°C. Membrane filter procedures were used to follow decreases in total and fecal coliform populations and total plate counts at each test pH and temperature. In both sewages, it was observed that pH values above 11 were required to reduce coliform populations to levels below 100/ml in less than 8-12 hours. To attain coliform population reductions to 1/ml or less, 24 hours were required at pH 11 but only 90 minutes at pH 11.5. Coliforms and other organisms concentrated in the precipitated solids during lime treatment; their numbers decreased as pH and/or contact time increased. Temperature was a less significant factor in the disinfection mechanisms than was pH.

An additional effect of lime treatment of sewage is the reduction of organic and inorganic chemical loads in the effluent. The reductions at 15°C for raw and 10°C for secondary treated, measured by BOD and orthophosphate tests, reached maximum BOD removals of 77 and 94%, respectively, at pH 11 in 24 hours for raw sewage and at pH 11.5 in 90 minutes for treated sewage. Likewise, maximum orthophosphate removals, 93 and 97%, respectively, were obtained at pH 12.0 for 60 minutes with raw sewage and pH 12.0 for 60 minutes for treated samples.

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CONTENTS

		Page
Abstract		ii
List of Figures		iv
List of Tables		vii
Acknowledgements		ix
Sections		
I	Conclusions	1
II	Recommendations	2
III	Introduction	3
IV	Materials and Methods	17
V	Experimental Results	24
VI	Discussion	76
VII	References	85

FIGURES

No.		Page
1	Effects of Lime and 3 N KOH on the pH of 2 liter Sewage Samples at 5°C	25
2	Effects of Lime on the pH of 2 liter Secondary Treatment Sewage Samples at 5°C	26
3	Effect of Adding Lime Slurry to Sewage, Followed by 3 N KOH, at 5°C	27
4	Reduction of Total Coliform Bacteria in Lime Treated Sewage at an Initial pH of 10.0, at 1 to 15°C	30
5	Reduction of Total Coliform Bacteria in Lime Treated Sewage at an Initial pH of 10.5, at 1 to 15°C	31
6	Reduction of Total Coliform Bacteria in Lime Treated Sewage at an Initial pH of 11.0, at 1 to 15°C	32
7	Reduction of Total Coliform Bacteria in Lime Treated Sewage at an Initial pH of 11.5, at 1 to 15°C	33
8	Reduction of Total Coliform Bacteria in Lime Treated Sewage at an Initial pH of 12.0, at 1 to 15°C	34
9	Reduction of Total Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 10.0, 10.5 and 11.0, at 10°C	35
10	Reduction of Total Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 10.0, 10.5 and 11.0, at 1°C	36
11	Reduction of Total Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 11.5 and 12.0, at 10°C	37
12	Reduction of Total Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 11.5 and 12.0, at 1°C	38
13	Reduction of Fecal Coliform Bacteria in Lime Treated Sewage at an Initial pH of 10.0, at 1 to 15°C	39
14	Reduction of Fecal Coliform Bacteria in Lime Treated Sewage at an Initial pH of 10.5, at 1 to 15°C	40

No.		Page
15	Reduction of Fecal Coliform Bacteria in Lime Treated Sewage at an Initial pH of 11.0, at 1 to 15°C	41
16	Reduction of Fecal Coliform Bacteria in Lime Treated Sewage at an Initial pH of 11.5, at 1 to 15°C	42
17	Reduction of Fecal Coliform Bacteria in Lime Treated Sewage at an Initial pH of 12.0, at 1 to 15°C	43
18	Reduction of Fecal Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 10.0, 10.5 and 11.0 at 10°C	44
19	Reduction of Fecal Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 10.0, 10.5 and 11.0, at 1°C	45
20	Reduction of Fecal Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 11.5 and 12.0, at 10°C	46
21	Reduction of Fecal Coliform Bacteria in Lime Treated Secondary Effluent at Initial pH's of 11.5 and 12.0, at 1°C	47
22	Survival of Total and Fecal Coliform Bacteria in Sewage Controls at 1 to 15°C	48
23	Survival of Bacteria in Secondary Effluent Controls at 1 and 10°C	49
24	Survival of Total and Fecal Coliform Bacteria in Sewage Control at 1 to 15°C	50
25	Survival of Bacteria in Secondary Effluent Controls at 1 and 10°C	51
26	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 10.0 through 11.0, at 1°C	64
27	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 11.5 and 12.0, at 1°C	65
28	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 10.0 through 11.0, at 5°C	66
29	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 11.5 and 12.0, at 5°C	67

No.		Page
30	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 10.0 through 11.0, Lime Treatment at 10°C	68
31	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 11.5 and 12.0, Lime Treatment at 10°C . .	69
32	Reductions in BOD and Orthophosphate Concentrations of Sewage at pH 10.0 through 11.0, Lime Treatment at 15°C	70
33	Reductions in BOD and Orthophosphate Concentrations of Sewage of pH 11.5 and 12.0, Lime Treatment at 15°C	71

TABLES

No.		Page
1	Back-titration of Neutral and High pH Sewages (2 liters each) with 2 M Calcium Chloride at 5°C . . .	28
2	Survival of Standard Plate Count Organisms (SPC) in the Supernatant Phase of Lime Treated and Control Sewages	52
3	Survival of Standard Plate Count Organisms (SPC) in the Supernatant Phase of Lime Treated and Control Sewage	53
4	Comparative Numbers of Total Coliform Bacteria Found in Sewage Supernatant and Precipitated Solids During Lime Treatment	54
5	Comparative Numbers of Fecal Coliform Bacteria Found in Sewage Supernatant and Precipitated Solids During Lime Treatment	55
6	Comparative Numbers of Standard Plate Count Bacteria Found in Sewage Supernatant and Precipitated Solids During Lime Treatment	56
7	Comparative Numbers of Total Coliform Bacteria Found in Secondary Effluent Supernatant and Sludge During Lime Treatment	57
8	Comparative Numbers of Fecal Coliform Bacteria Found in Secondary Effluent Supernatant and Sludge During Lime Treatment	58
9	Comparative Numbers of Standard Plate Count Bacteria Found in Secondary Effluent Supernatant and Sludge During Lime Treatment	59
10	Reductions of BOD and Orthophosphate Concentrations in Lime Treated Sewage at 1, 5, 10 and 15°C	61
11	Reductions of BOD and Orthophosphate Concentration in Lime Treated Secondary Effluent at 1 and 10°C . . .	63
12	Observed pH Changes in Lime Treated Sewage Samples Over the Contact Period	72
13	Observed pH Changes in Lime Treated Secondary Effluent Over the Contact Period	72

No.		Page
14	Observed Temperature Variations in Lime Treated Raw Sewage Samples Over the Contact Period	73
15	Observed Temperature Variations in Lime Treated Secondary Effluent Over the Contact Period	73
16	Average Quantities (ml) of Lime Slurry (132 mg/CaO/ml) Used to Produce Treatment pH Values in 2 liter Raw Sewage Samples	74
17	Average Quantities (ml) of Lime Slurry (117 mg/CaO/ml) Used to Produce Treatment pH Values in 2 liter Secondary Effluent Samples	74
18	Ranges and Means of the Measured Values of the Various Sewage Characteristics (5/3/71 to 12/2/71)	75

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SECTION I

CONCLUSIONS

Even in the presence of relatively high concentrations of organic matter and under the adverse conditions of cold temperature sewage can be disinfected to a safe level by lime treatment to pH 11.5 or 12.0.

A variety of generic types of bacteria can be destroyed during lime treatment, as evidenced by the large reductions in raw sewage, settled solids and treated sewage total and fecal coliform and total bacterial concentrations.

The process of disinfection can be completed within a very short time period (30 minutes or less), even at 1°C.

Effluents with greatly reduced concentrations of biologically oxidizable organic materials and orthophosphate can be produced, also in relatively short time periods (under 30 minutes).

SECTION II

RECOMMENDATIONS

Additional investigations should be directed at the effects of differing concentrations of organic matter on the rate of disinfection and the efficiency of organic matter and orthophosphate removals from raw and settled sewages with lime. Rudolfs and Gehm (62) indicated that primary settling may not be necessary, or desirable, for maximum removal of organic material by chemical precipitation. Therefore, there exists a need to determine the rate of disinfection in a system where organic matter concentrations could be varied under controlled conditions.

Further investigations could also be directed toward the determination of the form or forms of organic material remaining in effluents from lime treatment of sewage. Results of this study indicate that almost all of the large and finely divided suspended organic matter are removed by lime precipitation; however, a large portion of the biologically degradable material is represented by colloidal and soluble organics which lime treatment may not affect. Rudolfs and Gehm (63) stated that the soluble fraction of the BOD was least affected by chemical precipitation, which is probably the case with lime precipitation.

The results obtained in this study represent laboratory batch scale tests and probably do not provide adequate data for a flow-through system. Therefore, laboratory bench scale studies should be carried out with a flow-through system to determine disinfection rates and efficiency. The laboratory tests should be scaled up to pilot size in an operating domestic sewage collecting system. The efficiency of disinfection under these conditions would provide the needed operational design criteria.

Additional information is also needed on the affects of elevated pH on the survival of pathogenic viruses, nematodes and other aquatic life forms.

SECTION III

INTRODUCTION

When nomadic people began to form communities, sewage disposal became a problem and many such small isolated communities still exist today throughout the United States and the world. Geographically, many of these settlements are located in regions where severely cold climatic conditions prevail through much of the year. In these regions, the problems associated with sewage disposal have been intensified as the result of decreased biological decomposition of wastes at low temperatures. Where the communities have been unable to support public sewage treatment facilities because of the high costs involved in their construction and maintenance sewage disposal has been the responsibility of the individual homeowner.

In dealing with the problem to date, disposal methods have included the pit privy, septic and Imhoff tanks, lagooning systems, chemical treatment, abandonment on ice packs and, in some instances, direct outfall into waterways and estuaries. All of these disposal methods are unsafe from an epidemiological point of view because of the preservation of pathogenic organisms at cold temperatures. Concurrent with the recognition of danger to health we have become increasingly aware of environmental pollution and its association with inadequate sewage disposal practices. Therefore, in order to curtail the spread of disease and preserve our environment it is necessary to adequately disinfect and purify, to an acceptable level, all domestic sewages and associated wastes.

If the contamination from sewage can be contained by retention the adverse effects to the environment will be minimal. However, when natural biological decomposition is retarded, as is the case at low temperature, it is possible for the contamination to become dispersed over a large area with serious environmental degradation. The natural processes of eutrophication may be increased, previously used sources of domestic water may be rendered unfit for use, epidemiologic hazards increase, the ecology of an entire region may be severely upset and wildlife and recreational areas may be badly damaged or destroyed.

Because the methods of sewage treatment and disinfection currently employed by many small communities in cold regions are inefficient, there exists a need for modified, new or improved systems. This study is an endeavor to better understand the variables and relationships in a modification of a well known system.

Lime (CaO) treatment of domestic wastes will be studied for its bacterial disinfection capabilities along with attendant reductions in nutrient levels. Lime has been chosen because of its past record

of effectiveness in the clarification of domestic water supplies and precipitation of sewage solids, as well as its low cost.

OBJECTIVES

(1) To determine the effects of high pH on raw and treated sewage bacteria at low temperatures (1° to 15° C).
(2) To determine the degree of BOD removal from sewage at high pH and low temperatures.
(3) To determine the effectiveness of lime as a precipitant of orthophosphate ($o\text{-}PO_4$) from sewage at low temperatures.

LITERATURE REVIEW

Considerable literature has been published on the bactericidal effects of hydroxides, especially in the area of domestic water treatment (33,35,59,73,80). Likewise, ample information has been reported on the removal of suspended solids, BOD and phosphate compounds from sewage using both conventional biological treatment systems and physical-chemical methods (11,54,56,61,78). On the other hand, little information can be found relating the disinfection of sewage with the removal of suspended solids, BOD and phosphates, using alkalies in a single treatment procedure. Furthermore, most methods of sewage treatment are adversely affected by low operational temperatures (5,15,36,44) and insufficient information exists relating the effects of low temperatures on the clarification and disinfection of sewage with lime and other caustics.

The literature pertaining to this study will be reviewed under the following headings: Adverse effects of high pH on bacteria; Evaluation of the germicidal efficiency of hydroxides; Factors, other than high pH, affecting the bactericidal efficiency of hydroxides; Sewage disposal problems in the Arctic; Effects of low temperature on conventional sewage treatment systems; Removal of suspended solids, BOD and orthophosphate from sewage with lime.

Adverse Effects of High pH on Bacteria

Bacterial inhibition from caustic conditions has long been known. As early as 1878, Endemann (26) observed that "soda" was inhibitory to bacteria. It was later that Houston (35) first proposed the application of excess lime treatment to raw waters with the expressed purpose of removing bacteria of the "colon-typhoid" group. According to Houston, the first to apply the excess lime treatment in a water supply to destroy "plankton" development and produce an epidemiologically safe water was Accra in 1917. Eddy (22), investigating the same general problem, related the pH at which maximum precipitation of aluminum hydroxide occurs in alum treated waters with the "possible" control of growths in filter plants.

About the same time Hoover and Scott (34), while examining lime, softened water, discovered that bacterial tests were negative 92% of the time in 1 ml samples. Efforts to understand these findings led to the theory that the bactericidal action resulted from the presence of lime in the water. It was discovered that when enough lime was added to remove the free and half-bound carbonic acid and precipitate the magnesium, the bacteria of the "colon-typhoid" group were killed in 48 hours. Furthermore, the sterilizing action was found to persist indefinitely.

Additional data presented by Houston (35) indicated that lime treatment of raw river water resulted in large reductions in turbidity, color and hardness. In addition, no "Bacterium coli" could be found in 100 ml samples when the causticity of the effluent water was at least 5.0 mg/1. Edwards (23,24) reported that water with greatly reduced bacterial loads could be produced from heavily contaminated Ohio River water by application of the excess lime treatment. The Escherichia coli index was reduced from 20-40,000 per 100 ml to 100 per 100 ml when 20-25 mg/1 causticity was applied to the primary settling basins. During early investigations, Hoover (33) noted that lime treatment of raw water supplies enhanced suspended solids removal and that it was also possible to remove a higher percentage of potentially pathogenic microorganisms when excess lime treatment was used, 20 mg/1 yielded bacteriologically safe conditions in water mains.

Not everyone agreed that caustic conditions were necessary to produce water free of "colon" bacteria. Bahlman (4) found that effluents practically free of "colon" bacteria could be produced with "subcaustic" doses of lime. Investigations led to the conclusions that "coli" were actually killed by doses of lime which produced "caustic" conditions and were not merely removed by entrainment in the precipitated solids. On the other hand, exposure to low alkaline treatments was thought to result in physical removal (33).

By 1930, excess lime treatment of water supplies had been practiced for a number of years. Van Arnum (77) reported that waters containing low coliform concentrations had been produced for 11 years in Youngstown, Ohio, using the excess lime method. Streeter (73) studied some of the chemical effects of lime treatment on a raw water supply. He found that increases in bactericidal efficiency paralleled increasing degrees of causticity; furthermore, the application of the excess lime treatment (relatively high pH values) was capable of producing effluents which compared favorably to those produced by raw water prechlorination in combination with ordinary postchlorination. Streeter (73) was unable to confirm the findings of Bahlman (4) who reported bactericidal actions from "subcaustic" doses of lime.

Extensive studies on the survival of bacteria during the excess lime treatment of water were performed by Wattie and Chambers (80). They

concluded that the death rate of E. coli was very slow over a 10 hour contact period when exposed to a pH range of between 9.01-9.50 at 25°C. Increasing the pH range to 9.51-10.00 reduced the lag period to six hours and to three hours when the pH range was 10.01-10.50. In the pH ranges of 10.51-11.00 and 11.01-11.50, there were sudden drops in E. coli numbers in the first two to three hours. Two strains of Aerobacter aerogenes proved to be more resistant than the E. coli tested. Salmonella typhosa did not survive hydroxyl ion concentrations in the range of pH 11.01-11.50 longer than two hours, while Shigella dysenteriae was destroyed rapidly in all pH ranges studied; pH 11.01-11.50 produced 100% kill in 75 minutes.

Further confirmation of the efficiency of high pH came from Riehl, Hartung and Taylor (59) who demonstrated that during lime softening of raw river water, a bacteriologically safe water could be produced if the pH was kept above 11.4 for a minimum of three hours; however, the water was too caustic for domestic use.

Recent investigations on lime treatment of water supplies (58) indicated that addition of lime, sufficient to absorb the free and half-bound carbonic acid and precipitate the magnesium, reduced the "colon-typhoid" bacteria 99.67% in five hours. When an excess of one grain and three grains per gallon were added the action was 99.93 and 100% effective, respectively, in five hours.

Investigations directed toward the effects of high pH upon bacterial growth were not limited to those of the water treatment industry. Cohen and Clark (17) studied the growth of E. coli in media of high pH and indicated that there was a marked increase in the lag phase at a pH of about 8.9. Although the exact mechanism was unknown, it was theorized that acid or alkaline media may affect specific fermentative processes, specific rates of death and reproduction in quite different ways.

Scott and McClure (68) reviewed the available literature which indicated that bacteria of the "colon-typhoid" group were killed in media of low hydrogen ion concentrations. The data suggested that pH 9.5 was the lower limiting value in various media. They indicated that the limiting pH in the excess lime treatment of domestic water supplies was also 9.5. McCulloch (46) revealed that pH 10.00 was very germicidal against E. coli, S. typhosa and Salmonella paratyphosa, while a pH of 12.2 was required to produce the same action against Staphylococcus aureus.

Disinfectants have been commonly used in the agricultural industry for many years. Several investigators have evaluated high pH for its germicidal efficiency. Mudge and Lawler (50) concluded that sodium hydroxide solutions yielding pH values greater than 12.3 were very efficient bactericides. Bacteria from unwashed milk bottles, at a concentration of 8×10^6/ml, were completely destroyed in less than

four minutes, when the pH was adjusted with sodium hydroxide to 13.3-13.4 at 48.8°C. Myers (53) reported that in the course of sanitizing milk bottles, those washing powders that gave washing solutions of high pH were decidedly more effective as germicides than those that gave washing solutions of low pH. It was also shown that addition of sodium hydroxide to solutions of neutral pH greatly increased germicidal efficiency. Schaffer and Tilley (65) stated that soap solutions produced from neutral coconut oil and sodium hydroxide were much more effective against bacteria than those soaps that did not contain sodium hydroxide.

More recently, Le Compte (41) reported experimental results obtained from the lime treatment of wastewater from a paper processing mill. Addition of lime to effluent mill water resulted in the formation of a magnesium hydroxide precipitate which removed a large percentage of the suspended fiber material. In addition, the water from the excess lime treatment step was reported to have been "sterile" because of the high pH, and control over odors was achieved.

In the process of washing and transporting sugar beets, large numbers of bacteria have been reported to accumulate in the water. Schuyler (67) found that bacteria (predominately Leuconostoc mesenteroides) indigenious to sugar beet wastewater were not appreciably affected by lime additions resulting in pH values less than 11.2 at 25°C, while under the same set of conditions a laboratory strain of E. coli was rapidly destroyed.

Early reports on the use of lime in the treatment of sewage indicated that there were problems in evaluating the germicidal efficiency of lime. Data presented by an anonymous author (3) on sewage treatment with lime alone versus an electrolytic-lime treatment indicated that substantially better removals of E. coli were achieved with an electrolytic-lime system than with lime alone. Treatment pH values were not reported; however, the lime dosage was 3720 pounds per million gallons, electrolytic contact was 70 seconds, the total alkalinity of the effluents was 350 mg/l and the effluents were reported to be free of E. coli. A few years later, Lanphear (40) observed that there was no real advantage to an electrolytic-lime treatment because all effluents with total alkalinity values of 100 mg/l or more were bacteriologically stable without electrolytic treatment.

Results obtained from lime treatment of sewage were reported by Doyle (20) who indicated that the survival of S. typhosa was directly dependent upon the pH of sewage sludge filter cake. Furthermore, S. typhosa could not be cultured, even by enrichment procedures, when the pH of the filter cake was maintained above 11.00 for 24 hours.

Several viruses have been reported to be very susceptible to excess lime treatment. Thayer and Sproul (74) extensively researched the inactivation of T-2 phage during a variety of lime softening procedures.

T-2 phage was shown to be inactivated by lime additions resulting in a pH of about 9.2, with only 0.1% remaining active at pH 10.8. In addition, they stated that "many animal viruses are at least as pH sensitive as the T-2 bacteriophage" and that most viruses are unable to tolerate pH values above 11.0 and retain their infective activity. Van Vuuren et al. (78) found that by maintaining a concentration of 300 mg/l of hydrated lime in humus tank effluents, a polio virus ID_{50}/ml of zero could be produced when the contact time was 43 minutes. The E. coli concentration was observed to be reduced from 760/ml to 0/ml in the same contact period.

Evaluation of the Germicidal Efficiency of Hydroxides

The need to evaluate the germicidal efficiency of various hydroxides prompted investigators to determine the components responsible for their germicidal actions. Lamanna and Mallette (38) recognized the value of alkalies, asserting that they are efficient and economical disinfectants, the efficiency being a function of hydroxyl ion concentration. They stated that alkalies hydrolyze many proteins and nucleic acids at room temperature and sufficiently high hydroxyl ion concentrations will destroy organisms by direct reaction with physiologically active cellular constitutents and structures. Pelczar and Reid (57) concurred that the disinfectant action is dependent upon the dissociation of the alkali and the resulting hydroxyl ion concentration; however, they stated that lime has little, if any, activity as a disinfectant. In addition to the bactericidal action from the hydroxyl ion, many hydroxides have been reported to possess added bactericidal activity due to their cation (38,57).

Belief that low hydrogen ion concentrations (high pH) were responsible for the germicidal action of alkalies developed early in the application of the excess lime treatment to water supplies. Scott and McClure (68) reviewed the available literature, concluding that low hydrogen ion concentrations were responsible for bacterial reductions in lime treated water. McCulloch (46) also supported the theory that the germicidal action of alkalies, sodium hydroxide in particular, was primarily influenced by hydroxyl ion concentrations.

Levine et al. (41), however, stated that pH alone cannot be used as an index of the germicidal properties of different alkaline compounds, even though the germicidal efficiency of any particular alkali was shown to be a direct function of the hydroxyl ion concentration. For instance, the germicidal efficiencies of sodium carbonate, sodium phosphate and sodium hydroxide, at the same pH and temperature, were shown to be quite different. Likewise, Myers (52) concluded that the effects of pH cannot be compared when two or more buffer systems are used. Levine et al. (42) demonstrated that the presence of carbonate in commercial washing compounds enhanced the germicidal efficiency of sodium hydroxide in such compounds, although there was no appreciable affect upon the pH.

Additional investigations into the effects of salt additions to alkalies led Levine et al. (43) to conclude that sodium chloride, potassium chloride, sodium carbonate or sodium phosphate, when added to sodium hydroxide solutions, increased the germicidal efficiency of the hydroxide substantially. Myers (53) studied the interrelated effects of pH, osmotic pressure and buffer index upon the death rate of spores. He concluded that the death rate was accelerated by holding any two factors constant and increasing the third. The osmotic pressure had the least influence of the three factors studied.

Schuyler (67) noted a marked difference in the death rate of bacteria found in sugar beet wash water at pH 11.2 and 11.4. He suggested that two modes of destructive action may occur from high pH values produced by lime addition. Up to some critical pH the determining factor may be the hydroxyl ion concentration, while above this pH value, the determining factor may be the large concentration of undissociated calcium hydroxide resulting from the proportionately greater quantities of lime needed to attain such high pH values. It has been shown that almost twice as much hydrated lime ($Ca(OH)_2$) is needed to produce pH 11.4 as compared to pH 10.4. As a result of this, much of the lime (hydrated) remains undissociated at high pH values in the form of calcium hydroxide (59,67).

Factors, Other than High pH, Affecting the Bactericidal Efficiency of Alkalies

Many factors, other than high pH, have been shown to affect the germicidal efficiency of alkalies. The temperature at which bacteria are exposed to high pH must be considered in evaluating the germicidal efficiency of alkalies. Scott and McClure (68) recognized the influence of temperature on the efficiency of hydroxides and reported that increasing the temperature of softened water from 8° to 27°C resulted in a pH change from 10.32 to 9.89. It was therefore concluded that in cold weather the caustic alkalinity would have to be higher to produce a pH destructive to most bacteria.

Results obtained by Mudge and Lawler (50) also upheld the principle that increased germicidal efficiency paralleled increasing temperature, for a given alkali at a fixed pH value. Levine et al. (42) indicated that as treatment temperatures were increased, the discrepancy between the germicidal efficiency of sodium hydroxide washing compounds with and without carbonate also increased. Myers (52) also came to the conclusion that increased bactericidal powers paralleled higher temperatures at a fixed pH value. Tilley and Schafter (76) found that the germicidal efficiency of solutions of sodium carbonate, trisodium phosphate or mixtures of either of these with sodium hydroxide was enhanced by increased temperatures.

Watkins and Winslow (79) found that when sodium hydroxide was the disinfectant, the rate of bacterial death was temperature dependent

between 22° and 50°C. Wattie and Chambers (80) and Chambers and Berg (13) showed that the death rate of E. coli decreased as temperatures were lowered from 25° to 1°C at a fixed pH value. More recently, Schuyler (67) found that Leuconostoc mesenteroides suspended in sugar beet wash water was more resistant to high pH at 10°C than at 25°C.

Contrary to these findings, McCulloch (46) stated that the rate of disinfection produced by sodium hydroxide appeared to be practically independent of temperature changes between 2° and 25°C.

Subsequent work by McCulloch and Costigan (47) indicated that even though the activity of sodium hydroxide showed but little variation between 2° and 25°C, it was somewhat more active at 40°C. In complete agreement with McCulloch's findings, were those of Cohen (16), who showed that there was practically no difference in the death rate of E. coli at 0°, 10° and 20°C when exposed to dilute phosphate buffers. Further temperature increases up to 30°C resulted in an increase in the death rate of E. coli.

Another factor which has been shown to affect the bactericidal efficiency of alkalies is the quantity of organic matter present. Lamanna and Mallette (38) stated that the protection provided by "colloidal organic matter against chemical disinfection may be called a protective colloid effect." Because organic matter is of biological origin, there is interaction between the hydroxide and the organic matter, which essentially reduces the effective concentration of the hydroxide.

Early investigations of lime softened water (34) indicated that the bactericidal effectiveness of lime decreased in the presence of large concentrations of organic matter. McCulloch and Costigan (47) substantiated this fact when they showed that much more alkali was required to produce germicidal conditions when organisms were suspended in heavy suspensions of fecal matter and in broths as well. More recent investigations (20,58,78) also indicated that organic matter provides protection for bacteria against the bactericidal action of alkalies.

In contrast to evidence supporting the protective effects of organic matter, Tilley and Schaffer (76) found that the presence of chicken feces, skim milk or defibrinated horse blood had little effect upon the germicidal efficiency of sodium carbonate, trisodium phosphate or a mixture of either of these with sodium hydroxide.

In addition to the effects of temperature and organic matter, several other factors play an important role in determining the efficiency of alkalies. Bacterial load has been shown to influence the bactericidal efficiency of alkalies. Lang (39) found that dead cells in a medium apparently provided some type of protective effect for surviving organisms. Winslow and Falk (81) studied this effect and surmised

that the protection was due to the liberation of acidic materials from dead cells, which created a zone of lower alkalinity around viable cells.

Shaughnessy and Falk (69) reported that when viable cells were exposed to excessive concentrations of calcium, they underwent lysis liberating buffer-like substances into the medium. Subsequent investigations by Shaughnessy and Winslow (70) indicated that E. coli changed the pH of the surrounding medium by excretion of acid-like substances. Watkins and Winslow (79) found that large populations of bacteria were able to survive high pH values more efficiently than small populations. They theorized that bacteria excrete base neutralizing acids into the medium, creating a microenvironment of reduced alkalinity in the area immediately surrounding each cell. Apparently large populations were able to do so more efficiently.

The age of bacterial cultures has also been shown to affect the bactericidal efficiency of disinfectants (38,75). Recently, Grabow, Grabow and Burger (29) reported that physiologically active cells from humus tank effluents were much more susceptible to high pH than those cells that were in the stationary phase of growth. Watkins and Winslow (79) recognized the importance of culture age in determining the death rate of vegetative cells. They found that vegetative cells followed a logarithmic death curve when exposed to alkalinity and heat, while spores were found to die at an accelerated rate as time of exposure increased. Myers (53) also showed that the death rate of spores increased as the length of exposure to high pH increased.

Varying genera and species of bacteria respond to high pH differently. Cohen (16) reported that E. coli was more resistant to high pH than S. typhosa in distilled and tap water. Wattie and Chambers (80) performed extensive tests on the survival of various genera of bacteria exposed to high pH. They found that A. aerogenes was somewhat more resistant to high pH than E. coli, both of which were more resistant than either S. typhosa or S. dysenteriae. The work of Doyle (20) on lime treatment of sewage sludge filter cake confirmed the findings of Wattie and Chambers; E. coli survives longer at high pH than S. typhosa.

Relatively concentrated solutions of sodium and potassium hydroxide have been used in the isolation of acid-fast Mycobacterium tuberculosis bacilli (9,57); testimony to the extreme resistance of the acid-fast organisms to extremely high pH. Recently, Grabow, Grabow and Burger (29) reported a marked difference in the bactericidal action of lime against Gram negative, Gram positive and acid-fast bacteria. Gram negative organisms were found to be more susceptible to high pH than either the Gram positive or acid-fast organisms. The acid-fast organisms proved to be practically unaffected by the high pH. Maintaining a pH of 11.5 for 60 minutes resulted in complete destruction of all Gram negative bacteria, the primary survivors being bacterial spores.

The fact that the organisms of the coliform group (E. coli, A. aerogenes, etc.) are more resistant to high pH than the "colon-typhoid" group (Salmonella, Shigella, etc.) is important. By setting standards which predicate that sewage effluents must be very low in coliform populations, it may be possible to determine, within certain confidence limits, the relative safety of those effluents as far as the "colon-typhoid" organisms are concerned. Kehr and Butterfield (37) emphasized the value of the coliform test as an indicator of the possible presence of pathogenic bacteria, even when the coliform populations were very small. Doyle (20), on the other hand, stated that the presence or absence of E. coli in high pH systems should not be used as an indicator of the presence or absence of S. typhosa. Instead, determination and subsequent application of high pH values which are destructive to S. typhosa should be used.

Sewage Disposal Problems in the Arctic

According to Boyd and Boyd (8), "one of the most difficult problems of the North, especially in inland regions, is sewage disposal." A review of the early methods of sewage disposal along coastal regions of the Arctic (7) revealed that one of the most primitive and economical methods practiced was deposition of sewage in 55 gallon oil drums, transporting them to and abandoning them on the coastal ice. The development of such devices as "electric toilets" and fuel oil sewage-carrier systems were reported to show promise; however, operational problems made them somewhat undependable. Lagoons were said to be promising if "starter" cultures could be developed which would be enzymatically active at low temperatures.

Dickens (19) indicated that the primary method of sewage disposal in permafrost areas of northern Canada was the "pail" system, where the raw sewage was disposed on the open tundra or in local water courses. The use of insulated and heated septic tanks in arctic regions was reported to be somewhat successful; however, the effluents presented problems because of pathogenic organisms. The widespread use of water-carried sewage systems in arctic regions has been limited due to the excessive expense involved in preventing freeze-up (19). Furthermore, low temperatures in arctic regions were reported to greatly impair most methods of sewage treatment. Thus the use of leaching pits, septic tank disposal fields and the pit privy are relatively impractical in arctic regions.

The feasibility of sewage disposal within the icecap of Greenland was studied by Ostrum, West and Shafer (55). Careful examination of ice cores from the area surrounding the sewage sump indicated that the contaminated ice volume was about twice the volume of the waste-water discharged. Others (15) theorized that the use of such sewage sumps would preclude the future use of the area and could possibly undermine the camp in subsequent years. A report on waste disposal by Navy camps in polar regions (15) revealed that about half of these camps

did not treat their sewage at all before discharge. Of those that did, the most common treatments used included extended aeration, septic tanks and stabilization ponds.

Several investigators have emphasized the need to adequately disinfect all sewages before discharge into the environment (15,19). Pathogenic bacteria have been shown to remain viable for extended periods when exposed to extremely cold temperatures. The magnitude of the potential health problems that exist when untreated sewage is deposited on the frozen tundra was pointed out by Gordon and Babbott (27). They showed that shigellae and salmonellae survived frozen conditions in seeded stools left on the open trundra for 17 and 45 days, respectively. The evidence indicated that these bacteria are very resistant to the cold and other natural features of the arctic environment. Gastroenteritis was reported to be relatively common in the Arctic. Disease was generally thought to be associated with the lack of adequte water treatment and sewage disposal, along with unclean conditions from the lack of wash water. To further emphasize the durability of intestinal organims in arctic environments, a report by Carey (12) revealed that intestinal bacteria had survived for about 50 years in frozen stools left by the members of the Scott and Shackelton Antarctic expeditions of 1915-16. A recent study on a subarctic river by Gordon (28) indicated the extended survival of index organisms.

The need to adequately disinfect all domestic sewage in polar regions is obvious; however, the methods are not! Clark, Alter and Blake (15) reviewed the possible methods, most of which were reported to be unsuitable for use in arctic environments. A system of electrochemical disinfection was reported to hold promise; however, location near salt water was a prerequisite. McKinney (48) reported that the rate of chemical reactions is temperature dependent, chlorination being no exception. The activity of chlorine was shown to be extremely low at 0°C. As temperatures increased, the reaction rate increased rapidly resulting in about twice the rate of kill for each 10°C rise in temperature. Butterfield (10) indicated that low temperatures reduced the disinfection efficiency of free chlorine and monochloramine. Dickens (19) reported that temperatures below 10°C caused difficulties with gaseous chlorination because of the formation of chlorine hydrate crystals. Other proposed treatment procedures (20) required the use of expensive fuels or elaborate equipment making them impractical for use in small arctic communities.

Effects of Low Temperature on Conventional Sewage Treatment Systems

Ludzack, *et al*. (44) indicated that the temperature of activated sludge operations affected BOD and COD removals. Operation at 5°C resulted in 10% lower removals than those obtained at 30°C. Likewise, lower temperatures adversely affected flocculation characteristics. Keefer (36) reviewed the literature pertinent to the temperature and efficiency

of activated sludge systems and reported that very low temperatures may decrease the efficiency of the system.

Trickling filters are probably more adversely affected by cold temperatures than activated sludge systems. Benzie (5) stated that "trickling filters operate more effectively in warm rather than cold climates." Furthermore, severely cold Michigan winters were reported to have resulted in the formation of thick layers of ice on rotating distributors and filter bed media. Some filters were so severely iced that they had to be removed from service, reducing the efficiency of the plant to the status of a primary treatment system. Clark, Alter and Blake (15) concluded that if conventional sewage treatment systems are to be used in arctic regions, they will have to be housed in heated, well insulated buildings to insure maximum operating conditions and avoid freeze-up.

Removal of Suspended Solids, BOD and Orthophosphate from Sewage with Lime

Stauffer (72) reported that the first patent for a chemical process of sewage purification was issued to Deboissieu in 1762. Lime was the chemical most commonly used, probably because of its effectiveness and low cost.

The lime softening process for water supplies was developed in 1841 by Dr. Clark of Edinburg, Scotland (14) and is now referred to as the Porter-Clark process (58). The formation of calcium carbonate and magnesium hydroxide precipitates, in conjunction with high pH, results in the flocculation of suspended solids with improved settling. Because the same general chemical reactions take place in wastewater, the early use of the lime softening process as an aid in the removal of suspended solids probably led to the use of lime as an aid in coagulation of sewage solids. Early reports on sewage treatment in America indicate that lime was used extensively in this capacity (71).

Rudolfs and Gehm (60) found that there are two pH zones where optimum removal of suspended solids occurs; pH 2-3 on the acid side and pH 10-11 on the alkaline side. These pH zones were not fixed, but varied with such factors as dissolved oxygen concentration, quantity and type of trade wastes and the quantity of iron coagulants needed for maximum coagulation. Investigations into the relationship between turbidity, suspended solids and BOD (61) led to the general conclusion that decreasing turbidity paralleled increasing removals of suspended solids and BOD. The effects of raw sewage presettling on the efficiency of suspended solids removal during subsequent lime treatment was also studied (62). There was little difference in suspended solids removal using lime in conjunction with and without presettling. The coagulation of unsettled sewage was, in some instances, superior to that of presettled sewage.

Van Vuuren, et al. (77), using a combined system of lime softening and

microbubble aeration, found that the COD of settled sewage could be reduced from 600 mg/l to less than 100 mg/l. The system, when used in conjunction with recarbonation, resulted in a reduction of total dissolved solids, a valuable deterrent to the salinity problem. Buzzell and Sawyer (11) noted that lime treatment of domestic sewages resulted in 50-70% BOD removals and that the supernatant liquids were generally "clear and sparkling". Attempts to further reduce BOD values by activated sludge treatment were unsuccessful due to the low BOD values in those effluents. Recently, Mulbarger, Grossman and Dean (51) found that lime treatment of secondary effluents produced an average removal in suspended solids and total organic carbon of 97 and 51%, respectively.

In the last two decades considerable attention has been directed toward undesirable algal growths in many lakes and ponds. The general opinion has been that these algal blooms were the result of excessive nutrient concentrations, including orthophosphate, in water courses receiving sewage effluents (1,56,66).

Sawyer (64) stated that the ultimate goal in phosphate removal was the maintenance of less than 0.01 mg/l of inorganic phosphates in waters receiving sewage effluents. More recent investigations by Dryden and Stern (21) indicate that orthophosphate levels of 0.5 mg/l or less will not support the growth of algae.

Many systems of phosphate removal have been reported in the literature (54,66). The general consensus of opinion has been that a system of chemical precipitation followed by biological treatment (66) or straight lime precipitation (1,54) would be most desirable from the standpoint of both economics and efficiency. Owen (56) reported that soluble phosphates could be removed from sewage plant effluents by lime addition; pH 10.0 usually being sufficient to precipitate most of the soluble phosphates. Lime addition of 545 mg/l of sewage resulted in a pH of 10.5, total phosphates were lowered 77% when settled for one hour. Results obtained by Schmid and McKinney (66) indicated that total phosphates could be lowered as much as 80% by lime treatment. Albertson and Sherwood (1) discovered that orthophosphate was more difficult to remove, using lime treatment, than either the organic phosphates or the complex polyphosphates. Lime addition in a successful tertiary treatment system to reduce phosphate levels as well as residuals was reported by Culp (18).

The exact chemical reactions in the precipitation of phosphate compounds with calcium hydroxide (hydrated lime) are not known. However, it has been shown that orthophosphate combines with calcium and magnesium at pH values above 10.5. The compounds formed closely approximate the hydroxyapatites of calcium $[Ca_3(PO_4)_2]_3$. $Ca(OH)_2$ and magnesium $[Mg_3(PO_4)_2 \cdot Mg(OH)_2]$, which are only slightly soluble at pH > 10 (45).

Buzzell and Sawyer (11) also recognized the importance of limiting

the phosphate concentrations in the control of excessive algal blooms. Results obtained from their investigations of phosphate removal from sewage with lime indicated that a maximum phosphate removal of 97% was obtained at a pH of about 11.0, while maximum precipitation of suspended solids occurred at pH 10.5. Proportionately greater concentrations of lime were required to attain pH values greater than 11.0 which resulted in calcium carbonate turbidity and little additional orthophosphate removal. In a comprehensive report on nutrient removal from wastewater, Eliassen and Tchobanoglous (25) stated that orthophosphate concentrations could be reduced to the level of 0.5 mg/l using lime coagulation-sedimentation and that chemical recovery was possible by calcination of the sludge.

Effluents from lime treatment systems are relatively caustic (59) and recarbonation of the high pH effluents has been practiced in the past. Lecompte (41) reported using scrubbed boiler flue gas, while Van Vuuren, et al. (78) used a source of gaseous carbon dioxide; however, they too recommended the use of flue gases. Mulbarger, Grossman and Dean (51) indicated that the carbon dioxide liberated from the calcination of the sewage sludge could be used in the recarbonation of effluents from the lime treatment system. Other methods of recarbonation include the use of alum and carbonic acid generated from coke, nitric cake solutions and sodium bicarbonate, were described by Houston (35); however, the use of gaseous carbon dioxide was superior to the other methods.

SECTION IV

MATERIALS AND METHODS

ANALYSIS OF LIME AND LIME SLURRY PREPARATION

Commercial grade lime was obtained locally and analyzed for percentages of calcium oxide (CaO), magnesium oxide (MgO), iron and aluminum oxides (Fe_2O_3 and Al_2O_3), silicon oxide (SiO_2) and loss on ignition by the Ideal Cement Co., Fort Collins, Colorado.

Analyses	I Raw Sewage Studies	II Secondary Treatment Studies
CaO	73.20%	65.20%
MgO	0.42%	0.47%
Al_2O_3 and Fe_2O_3	0.39%	0.15%
SiO_2	0.10%	0.40%
Loss on ignition	25.65%	32.60%
Total	99.76%	98.82%

Loss on ignition represents the percent $CaCO_3$, therefore the lime used in this study was strongly carbonated as the result of contact with atmospheric carbon dioxide.

The slaked lime slurry contained: I., 180 mg of lime per milliliter, with an available calcium oxide content of 132 mg per milliliter; II., 180 mg lime/ml and 117 mgCaO/ml. As contact with atmospheric carbon dioxide progressed, the appearance of solid calcium carbonate became apparent and the unused portion was discarded after one month.

To prepare the lime for use, it was slaked (mixed) with boiled, deionized water to produce a viscous slurry which could be pipetted, but concentrated enough to produce high pH values with minimal sample dilution. A slurry composition of five parts deionized water plus one part powdered quicklime (v/w) was found to be best suited for this study (each milliliter of slurry contained approximately 0.2 g of lime). Approximately 600 g of lime slurry were prepared and stored tightly sealed in a one liter polypropylene container until needed. The unused portion was discarded at the end of one month.

PREPARATION OF SEWAGE SAMPLES FOR TITRATION AND HIGH pH STUDIES

Throughout this study, sewage samples were collected at approximately 9:00 A.M. in an attempt to increase sample uniformity and minimize variations brought about by changes in flow rate, sewage composition, etc. Sampling was postponed for a 24 hour period after rain or snow.

All raw samples used in this study were collected from the Fort Collins trickling filter sewage plant #1 as the sewage left the grit removal chamber. Treated sewage samples were collected at the #2 treatment plant, an activated sludge unit, and taken from the secondary settling tank. Polypropylene containers (20 l), chlorine sanitized and then thiosulfate neutralized (2) were used for sample collection. Delivery to the laboratory required less than 15 minutes.

Ambient air and sewage temperatures were measured at the sample site with a Weston dial thermometer (± 0.5°C). In addition to the 20 liter sample, two 300 ml biochemical oxygen demand (BOD) bottles were carefully and completely filled with raw sewage and securely stoppered for rough estimation of the dissolved oxygen concentration upon delivery to the laboratory.

Immediately upon arrival at the laboratory, the 20 liter sewage sample was thoroughly mixed by shaking the container for one minute. The sewage was then decanted into five sterile 3 liter Erlenmyer flasks, capped with aluminum foil and placed in a -20°C freezer for settling of suspended solids and cooling.

For lime treatment at 1° and 5°C, the sewage was settled and cooled for one hour, after which the supernatant was aseptically siphoned into sterile 3.8 liter containers for additional cooling to the desired treatment temperature. The treatment containers were then removed from the cooling unit and placed in a temperature controlled environmental room, preset to either 1° or 5°C for treatment. For lime treatment at 10° and 15°C, the sewage was settled at -20°C until the desired treatment temperature was attained. The containers were then removed from the cooling unit and placed in an environmental room, preset to 10° or 15°C, to complete one hour of settling. After settling, the supernatant was siphoned into sterile 3.8 liter containers for treatment.

Before lime treatment, the contents from all containers were thoroughly intermixed to obtain sample uniformity. The final volume in each container was adjusted to two liters, the excess from each container being decanted back into the 20 liter polypropylene container for sterilization before discard. Each treatment container was covered with aluminum foil and transferred to a six-place magnetic stirring apparatus (Lab-Line Instruments, Inc.), located in the environmental room for subsequent treatment at the selected temperature.

TITRATION OF SEWAGE WITH LIME, POTASSIUM HYDROXIDE AND CALCIUM CHLORIDE

Titration of raw, settled sewage with lime was performed at temperatures of 5°, 10° and 15°C. Samples were continuously mixed during titration on a magnetic stirrer. All samples of sewage were mixed in this manner at a fixed speed. Prior to titration, the temperature and pH of the sample were determined using a Tele-Thermometer model 43TZ (Yellow Springs Instrument Co., Inc.) and an Accumet 220 pH meter (Fisher Scientific Co.), respectively.

During titration, prepared lime slurry was added to the sewage samples in increments large enough to produce a pH change of between 0.1 and 0.8 pH units. Successive lime additions and pH measurements were continued until a pH value of approximately 12.3 was attained. Duplicate sewage samples were titrated with lime slurry in this manner, at each temperature, and the results averaged.

Using potassium hydroxide (commercial grade - 85% maximum KOH), duplicate two liter sewage samples were titrated at 5°C and the results averaged into one titration curve.

A single titration curve was determined for a two liter sewage sample at 5°C, using a combination of lime to about pH 10.0, followed by the addition of 3 N potassium hydroxide to a pH of 12.3. Again, the procedure was the same as that used during the titration of sewage with lime slurry.

The pH of two, two liter sewage samples was adjusted to approximately 12.3, one with lime slurry and one with 3 N KOH, as described. Each sample was then back-titrated using a 2 M calcium chloride solution added in 10 ml aliquots and the resulting pH was measured. The effects of calcium chloride on high pH values as produced from lime and potassium hydroxide additions were determined at 5°C. The effects of a 2 M calcium chloride solution on the pH of an untreated sewage sample (pH 7.4) at 5°C were also determined, in the same manner.

LIME DISINFECTION OF SEWAGE BACTERIA AT COLD TEMPERATURES

Two liter sewage samples were prepared as described above. To determine the disinfection rate at the various pH values, two treatment procedures were followed because widely varying contact periods were necessary at initial treatment pH values of 10.0, 10.5 and 11.0 as compared to contact periods at initial pH values of 11.5 and 12.0. Therefore, they will be described separately.

Disinfection at pH 10.0, 10.5 and 11.0

Four, two liter samples, pH 10.0, 10.5, 11.0 and control, respectively, were thoroughly mixed for five minutes after which a 10 ml aliquot was withdrawn from each container for zero time bacterial enumeration. All samples were withdrawn with a sterile pipette and placed in sterile, labeled 25 x 150 mm screw-cap test tubes.

The temperature and pH of each treatment container was measured and the ambient air temperature in the controlled environmental room was continuously recorded with a Hydro-Thermograph model 594 (Freiz Instrument Div.).

Starting at time zero, with continued mixing, lime was added to the containers, adjusting the sewage pH to the desired treatment value. The control container recieved no lime. All containers were allowed to

mix for a total period of five minutes, after which each was allowed to quiescently settle. At time intervals of 2, 4, 6, 8, 10, 12, 18, 24, 36 and 48 hours, samples of 10-30 ml were aseptically withdrawn from each high pH container for bacterial enumeration. A sample of the control sewage was likewise removed at 48 hours. The temperature and pH of the contents in each container were measured each time a sample was withdrawn. All samples were withdrawn from the containers as close to the center and five centimeters below the sewage surface as possible. Three sewage samples collected on different days were exposed to these high pH values at 1°, 5°, 10° and 15°C, and the results obtained at each pH and temperature were averaged. All quantities of lime used to produce high pH values were recorded.

Disinfection at pH 11.5 and 12.0

Two liter samples were thoroughly mixed for five minutes, after which a 10 ml aliquot was withdrawn from each for zero time bacterial enumeration. The temperature and pH were measured before lime additions were made. Starting at time zero, with continued mixing, lime slurry was added to the containers adjusting the pH to the desired treatment values of 11.5 and 12.0. As before, the control received no lime. After a total mixing period of five minutes, the contents of each container were allowed to quiescently settle. Over a 90 minute contact period, 50 ml aliquots were aseptically withdrawn from each container at 10 minute intervals for bacterial enumeration. The control was sampled after 90 minutes of contact. As before, the temperature and pH were measured each time a sample was removed. Three sewage samples collected on different days were exposed to these high pH values at 1°, 5°, 10° and 15°C, and the results obtained at each pH and temperature were averaged.

PREPARATION OF PRECIPITATED SOLIDS FOR BACTERIAL ENUMERATION

The examination of precipitated sludge produced in all five high pH treatments was performed identically, except for the ranges of dilutions needed for bacterial enumeration.

At the termination of 48 hours of exposure to pH values of 10.0, 10.5 and 11.0, a 10 ml aliquot of the precipitated material from each container was aseptically removed, using a wide bore pipette, and placed in a separate sterile microblender with 90 ml of sterile phosphate buffered (0.02 M) dilution water. Likewise, at the end of 90 minutes contact with pH values of 11.5 and 12.0, a 10 ml aliquot of the precipitated material from each container was prepared. Each sample of precipitated material was immediately blended at high speed for 30 seconds and allowed to quiescently settle for five minutes. Serial 10-fold dilutions were then prepared from the supernatants of each to cover the expected range of bacterial concentrations found in the solids. In some cases, it was necessary to membrane filter 5 ml, 10 ml or even larger quantities of the supernatant, without preparing dilutions, in order to obtain countable plates.

ENUMERATION OF TOTAL COLIFORM, FECAL COLIFORM AND TOTAL PLATE COUNT BACTERIA

Difco media, used exclusively throughout this study, were freshly prepared, as needed, according to the manufacturer's specification. Total coliform bacteria were grown on m-Endo Broth M F, fecal coliform bacteria on m-F C Broth (plus rosolic acid) and the total plate counts were enumerated on m-Plate Count Broth. All media were stored at refrigerator temperature.

Enumeration of total and fecal coliforms were performed on the various samples immediately after being removed from the treatment container by the membrane filter method described in Standard Methods (2). Total plate counts, also called standard plate counts, were enumerated by a Millipore procedure adopted by this laboratory (67).

Bacterial counts in this study are reported in numbers per milliliter rather than the usually used numbers per 100 ml because of the high bacterial loading of the waste material studied.

All samples, other than precipitated sludge, were first thoroughly mixed for 30 seconds in sterile tubes with a vortex mixer. Immediately after mixing, serial 10-fold dilutions were prepared from each, anticipating the range of bacterial concentrations. Each dilution was then membrane filtered and washed three times with approximately 35 ml of sterile phosphate buffered (0.02 M) dilution water.

White-grid 0.45 μ Millipore membrane filters were used for enumeration of total and fecal coliform bacteria, while black-grid membranes were used for total plate counts. Incubations were according to Standard Methods (2) as follows: fecal coliforms, 44.5 $\pm$ 0.2°C for 24 hours with the plates enclosed in Whirl-Pac plastic bags submerged in a water bath; total coliforms, 35 $\pm$ 0.5°C for 24 hours; total plate counts, 20°C for 48 hours. Colony counting was under a 10-power stereoscope.

REDUCTIONS IN NUTRIENT LEVELS OF SEWAGE WITH LIME

Raw and secondary treatment sewage was collected and prepared as described previously, except that sterile conditions were not maintained.

The biochemical oxygen demand (BOD) of a 100 ml aliqout of raw sewage which had been homogenized in a microblender for 15 seconds was determined in duplicate using the azide modification (2). Likewise, the BOD of an aliquot of the supernatant from the same sewage settled for one hour was determined in duplicate. The orthophosphate concentration in fresh and settled samples was determined by the vanadomolybdate colorimetric technique, as described in Standard Methods (2).

Sewage samples settled as described earlier were thoroughly mixed on a six-place magnetic stirring apparatus. The pH of the sewage in five of the containers was then adjusted with lime to values of 10.0, 10.5, 11.0, 11.5 and 12.0, respectively, and another container was the control. All samples were continuously mixed for five minutes during lime addition with pH monitoring. Then the sewage in each container was allowed to quiescently settle.

Residual BOD and orthophosphate concentrations were determined in the supernatants of the lime treated sewages at the end of contact periods at the various temperatures, as outlined in the protocol.

Residual BOD and orthophosphate concentrations in lime treated sewages were determined on samples of sewage acquired on three separate days at temperatures of 1°, 5°, 10° and 15°C as previously described, and the results obtained at each pH and temperature were averaged.

Protocol for BOD and Orthophosphate

Protocol		Treatment pH values	
Temp. (°C)	Characteristic measured	10.0, 10.5, 11.0 and control	11.5, 12.0 and control
1	BOD	48 hours	90 minutes
	$o\text{-}PO_4$	24 hours	90 minutes
5	BOD	48 hours	60 minutes
	$o\text{-}PO_4$	24 hours	60 minutes
10	BOD	48 hours	60 minutes
	$o\text{-}PO_4$	48 hours	60 minutes
15	BOD	24 hours	60 minutes
	$o\text{-}PO_4$	24 hours	60 minutes

MEASUREMENTS OF PHYSICAL AND CHEMICAL SEWAGE CHARACTERISTICS

Measurements of the physical and chemical characteristics of many of the sewage samples were performed.

Tests

Settleable Solids - from a well mixed container of raw sewage, one liter was decanted into an Imhoff cone and allowed to stand upright for one hour. Settleable solids were determined and recorded as milliliters per liter of raw sewage

Suspended Solids - 100 ml of settled sewage (one hour) were filtered through asbestos in Gooch crucibles, evaporated to dryness and reported as mg/100 ml (2)

Conductivity - measured directly at 20°C on a sewage sample with a conductivity meter model RA-2A (Industrial Instruments, Inc.)

Turbidity - determined with a Klett Colorimeter (Summerson Photoelectric Colorimeter model 800-3), with a Blue #42 filter

Orthophosphate - determined using both the aminonaphtholsulfonic acid and the vanadomolybdate colorimetric techniques (2)

Calcium Hardness - determined in accordance with the procedure described in the Hach Chemical Company's Methods Manual, 7th Edition (31)

Total Alkalinity - measured by Hach sulfuric acid titration (31)

pH - Accumet 220 pH meter (Fisher Scientific Co.) at 20°C

Temperature - measured directly using a Weston dial thermometer

Dissolved Oxygen Concentration - determined in accordance with the modified azide technique as described in Standard Methods (2)

Biochemical Oxygen Demand - by the azide modification of Standard Methods (2).

SECTION V

RESULTS

Investigations into the use of lime as a low temperature chemical sewage treatment process were performed in phases in order to obtain a better understanding of the potential of such a system: Titration of Sewage with Lime, Potassium Hydroxide, and Calcium Chloride; Lime Disinfection of Sewage Bacteria; Reductions in the Biochemical Oxygen Demand (BOD) and Orthophosphate Concentrations of Sewage with Lime; Measurements of Temperature, pH and Quantities of Lime Added to Sewage; Measurements of the Physical and Chemical Characteristics of Raw Sewage Samples.

TITRATION OF SEWAGE WITH LIME, POTASSIUM HYDROXIDE, AND CALCIUM CHLORIDE

The data in Fig. 1 shows the general relationship between the quantity of lime slurry added to a two liter settled raw sewage sample and the resulting pH values at 5°C. Similar results were obtained at 10° and 15°C; however, more lime was required to produce pH values above 11.0 at 10° and 15°C than was required to produce equivalent pH values at 1° or 5°C. Figure 2 shows comparable pH increases when lime slurry was added to secondary treated sewage at 5°C.

Investigations into the use of potassium hydroxide to raise the pH of a two liter settled raw sewage sample are also shown in Fig. 1. The titration curve produced with potassium hydroxide was very similar to that produced with lime slurry, except at pH values above 9.3, where proportionately greater quantities of potassium hydroxide were required, compared to lime, to produce an equivalent pH change.

Investigations into the use of lime in conjunction with potassium hydroxide to reduce the quantity of lime needed in raw sewage to produce pH values above 10.0 are shown in Fig. 3. The titration curve was very similar to that produced when titrating with either lime or potassium hydroxide individually.

The pH of a two liter settled raw sewage sample was adjusted to 12.12 with lime at 5°C. Using a 2 M $CaCl_2$ solution, this sewage was then back-titrated to detect any pH repression. Addition of 50 ml of $CaCl_2$ solution resulted in a pH repression of 0.67 units; 50 ml to an equivalent two liter sewage sample adjusted to pH 12.00 at 5°C with 3 N KOH resulted in a pH repression of only 0.14 units. Furthermore, an additional 50 ml of 2 M $CaCl_2$ did not affect the pH of this sewage significantly; 50 ml in a two liter settled sewage sample at pH 7.58 (untreated raw sewage) resulted in a pH repression of 0.28 units (Table 1).

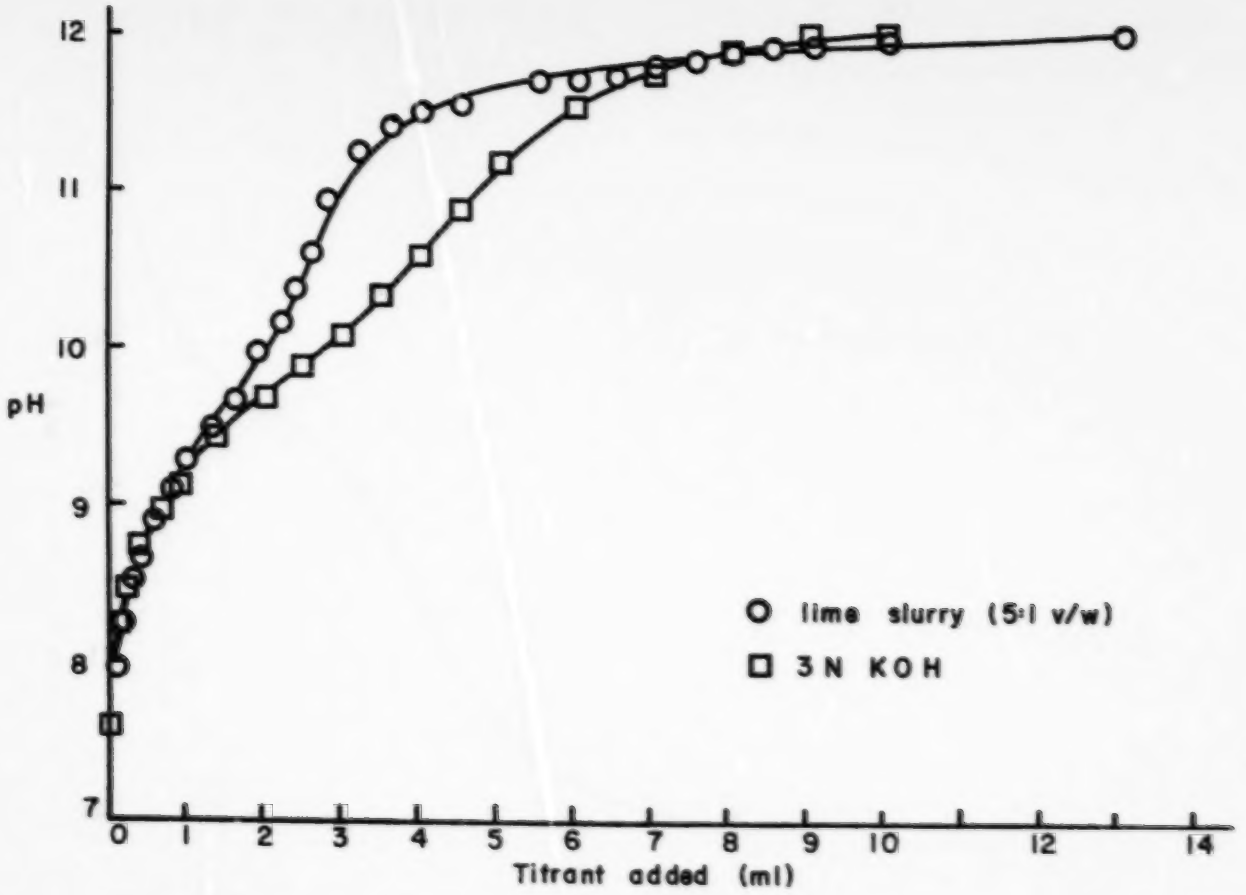

FIG. 1. Effects of lime and 3N KOH on the pH of 2 liter sewage samples at 5° C.

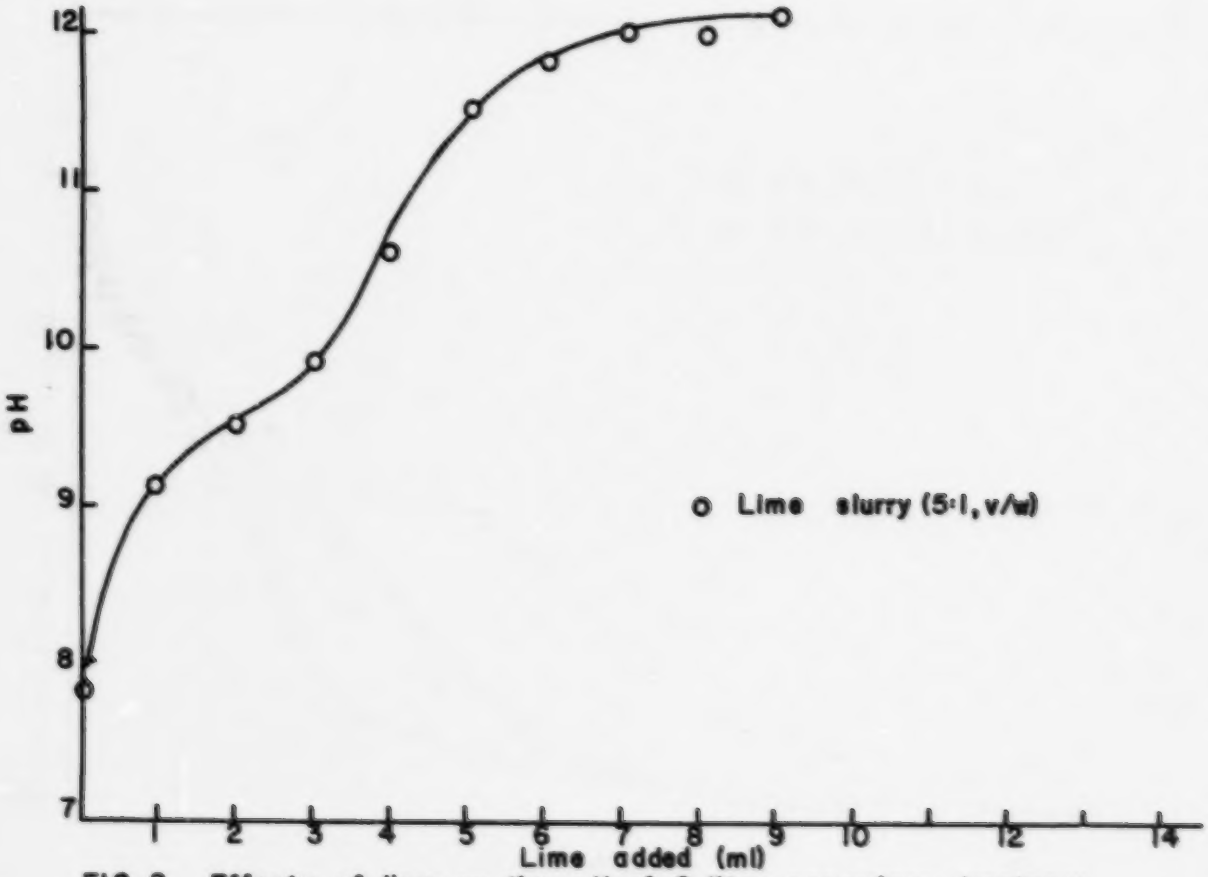

FIG. 2. Effects of lime on the pH of 2 liter secondary treatment sewage samples at 5°C.

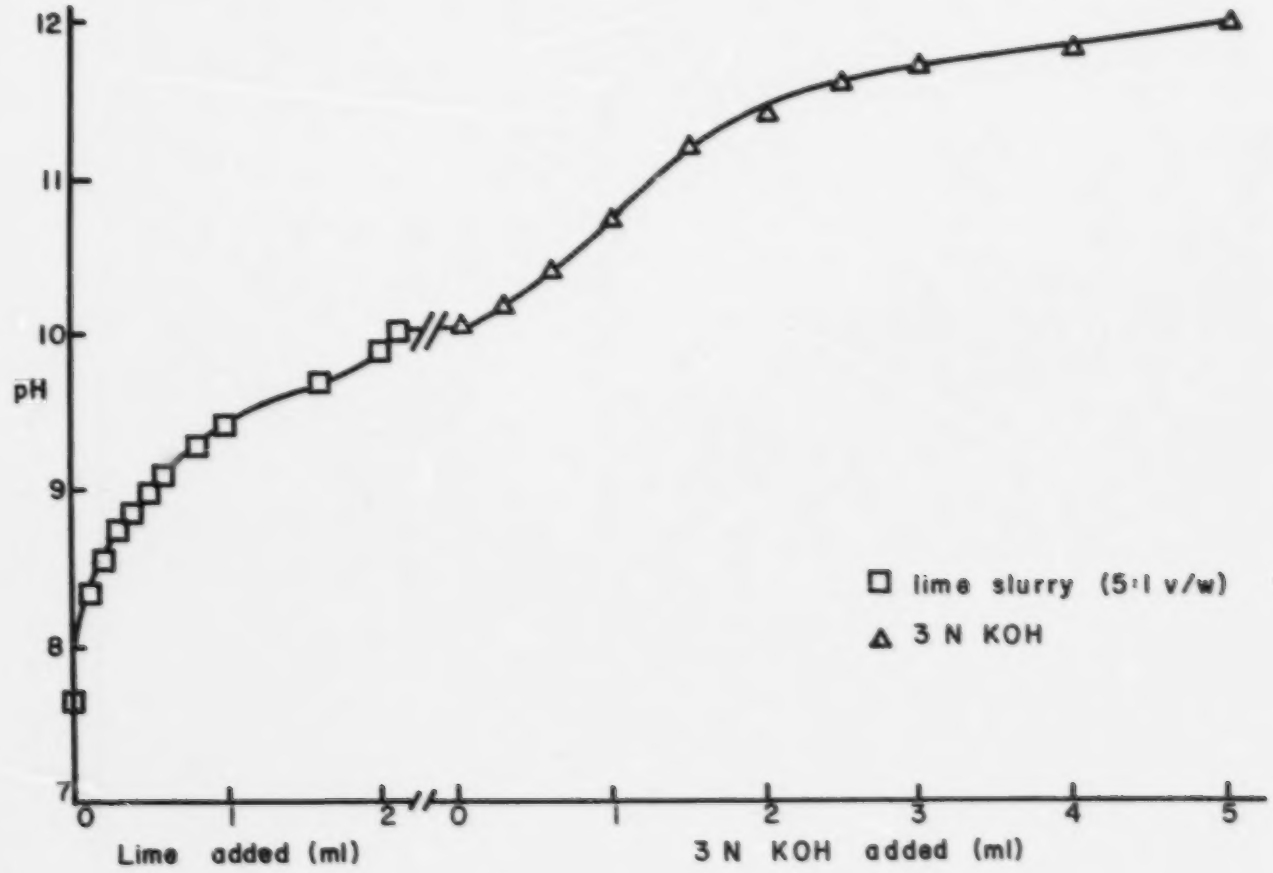

FIG. 3. Effect of adding lime slurry to sewage, followed by 3 N KOH, at 5° C.

Table 1. Back-titration of neutral and high pH sewages (2 liters each) with 2 M calcium chloride at 5°C.

Calcium chloride added (ml)	pH values 9/8/71		
	Lime treated sewage	KOH treated sewage	Control sewage
0	12.12	12.00	7.58
1.0			7.52
2.0			7.53
3.0			7.49
4.0			7.48
5.0			7.47
6.0			7.46
7.0			7.45
8.0			7.44
9.0			7.43
10.0	11.91	11.96	7.42
20.0	11.82	11.92	
30.0	11.63	11.87	
40.0	11.57	11.85	
50.0	11.53	11.82	7.30
60.0	11.50		
70.0	11.50		
100.0	11.45		

LIME DISINFECTION OF SEWAGE BACTERIA

The effects of temperature and an initial pH of 10.0 on the survival of total coliform bacteria in raw sewage are shown in Fig. 4. Successive increases in the pH by one-half unit and the resulting reductions in the total coliform concentrations are shown in Figures 4 through 8. Comparison of the results in these figures shows the parallel between increasing pH values and accelerated rates of total coliform mortality and a sharp breakpoint in the rate of coliform death at a pH of about 11.5. Figures 9 through 12 show the reduction of total coliforms in lime-treated secondary effluent at 1° and 10°C.

The effects of treatment temperatures and pH values on the survival of fecal coliform bacteria are shown in Figures 13 through 21. Although the initial concentrations of fecal coliform bacteria were generally one log unit lower than total coliform concentrations, the mortality rates of both groups were very similar. This is clearly shown by comparing the data for total coliform death with the corresponding data for fecal coliform death at the same pH and temperature.

In order to determine if any coliform death resulted from factors other than high pH, sewage controls were prepared and treated in the same manner as the high pH sewages except that no lime was added. Figures 22-23 show the survival of total and fecal coliform bacteria in controls held at low temperatures for 90 minutes. Likewise, survival of total and fecal coliform bacteria and standard plate count organisms in sewage controls at low temperatures for 48 hours are shown in Figs. 24-25. It is of interest to note that the data in Fig. 24 also shows that both total and fecal coliform bacteria were capable of growth in sewage controls at 15°C, but not at or below 10°C.

In order to obtain a better understanding of the effects of high pH on the survival of bacteria, total plate counts were performed initially and at the end of each high pH treatment. Tables 2 and 3 show the results obtained from such high pH treatments at the various temperatures, and from untreated sewage controls.

The possibility that large concentrations of organic matter affords protection to bacteria from the toxic effects of high pH was also investigated. Comparative counts of total and fecal coliform and standard plate counts in the supernatant and precipitated solids were performed at the end of each high pH treatment in order to determine this. The data is presented in Tables 4 through 9.

REDUCTIONS IN THE BIOCHEMICAL OXYGEN DEMAND (BOD) AND ORTHOPHOSPHATE CONCENTRATIONS OF SEWAGE USING LIME

Along with the destructive action of high pH against bacteria, sewage solids and orthophosphate were simultaneously removed during

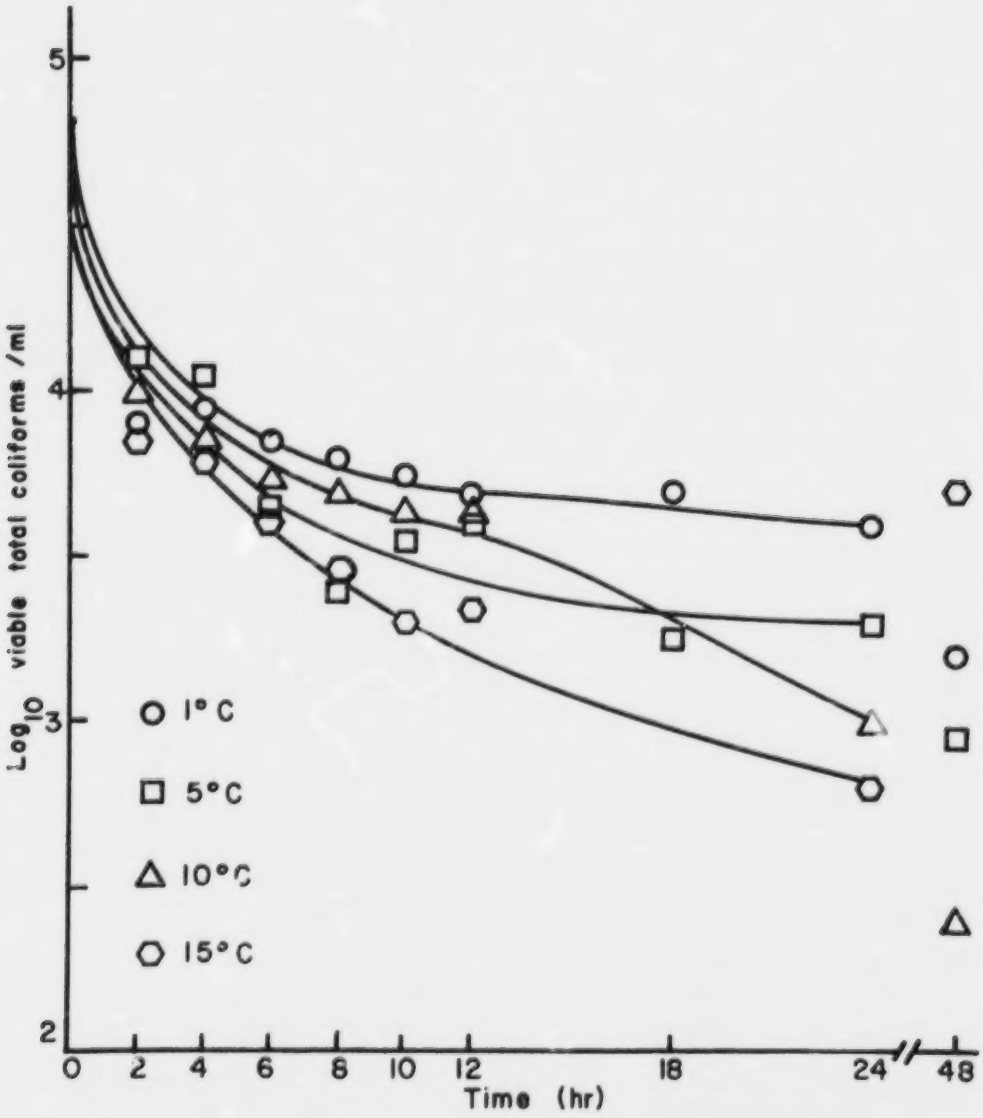

FIG. 4. Reduction of total coliform bacteria in lime treated sewage at an initial pH of 10.0, at 1 to 15° C.

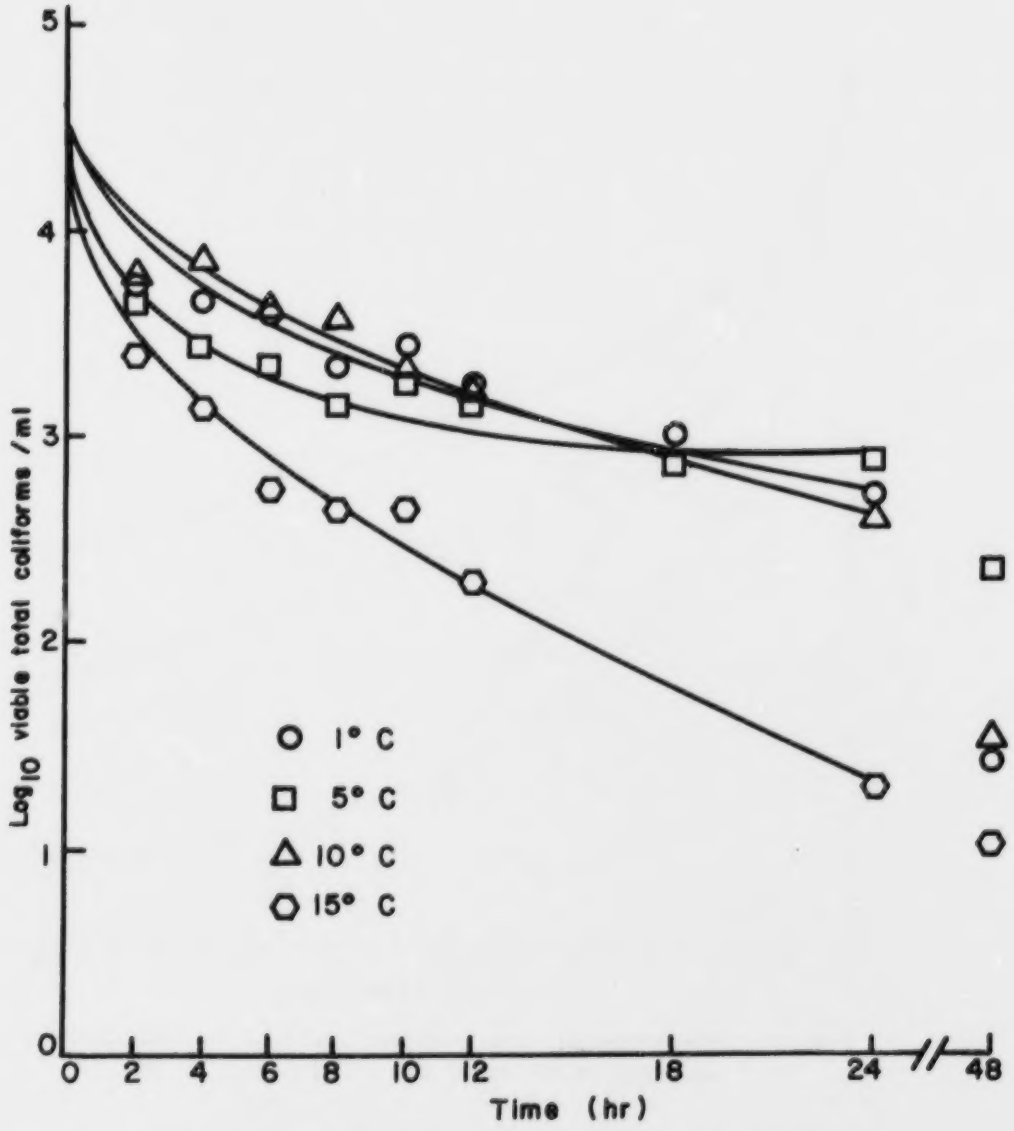

FIG. 5. Reduction of total coliform bacteria in lime treated sewage at an initial pH of 10.5, at 1 to 15° C.

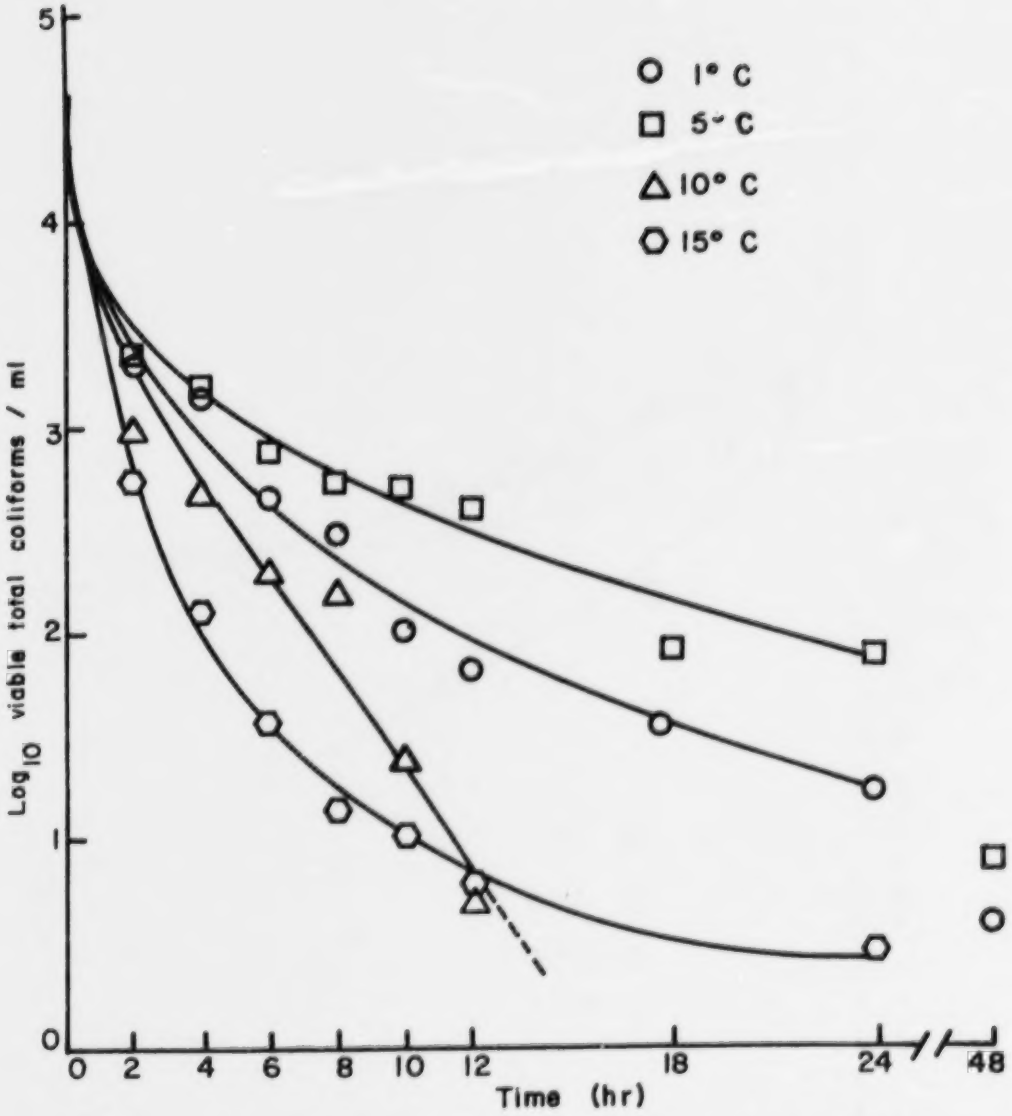

FIG. 6. Reduction of total coliform bacteria in lime treated sewage at an initial pH of 11.0, at 1 to 15° C.

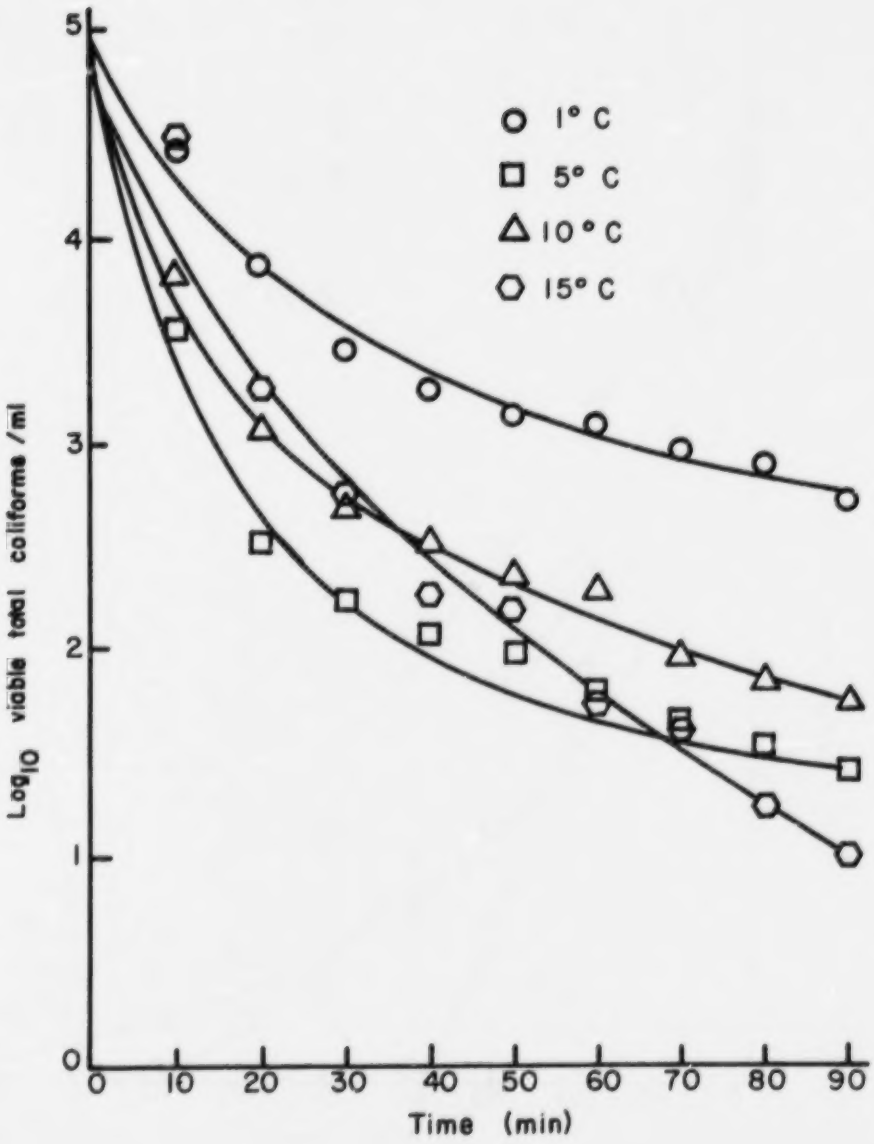

FIG. 7. Reduction of total coliform bacteria in lime treated sewage at an initial pH of 11.5, at 1 to 15° C.

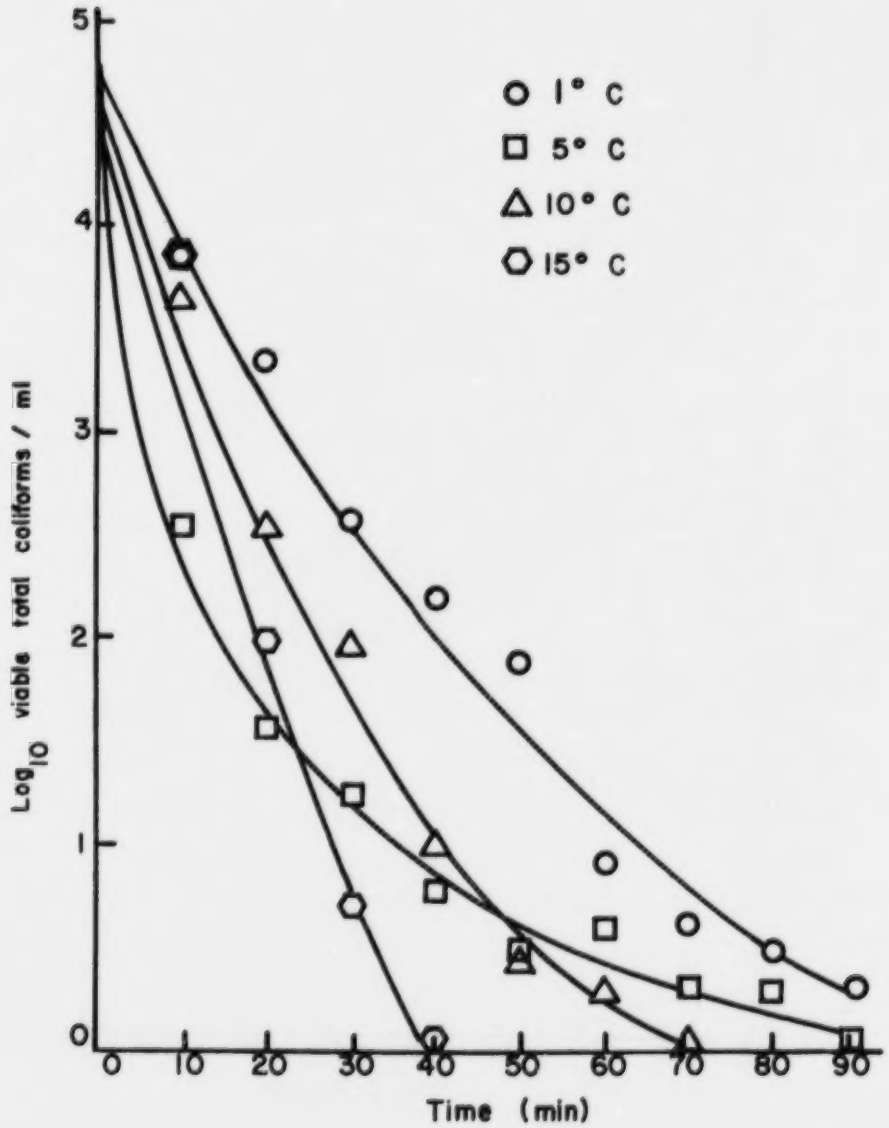

FIG. 8. Reduction of total coliform bacteria in lime treated sewage at an initial pH of 12.0, at 1 to 15° C.

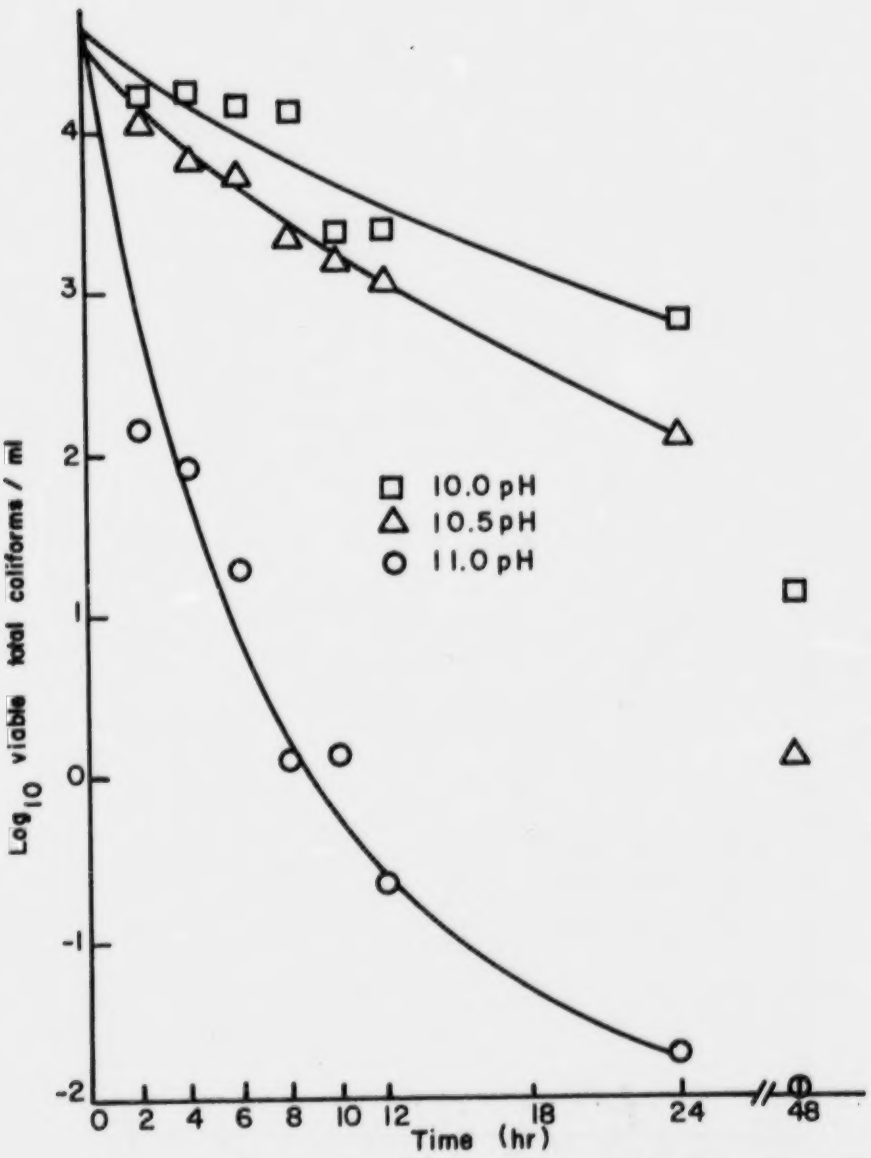

FIG. 9. Reduction of total coliform bacteria in lime treated secondary effluent at initial pH's of 10.0, 10.5 and 11.0, at 10° C.

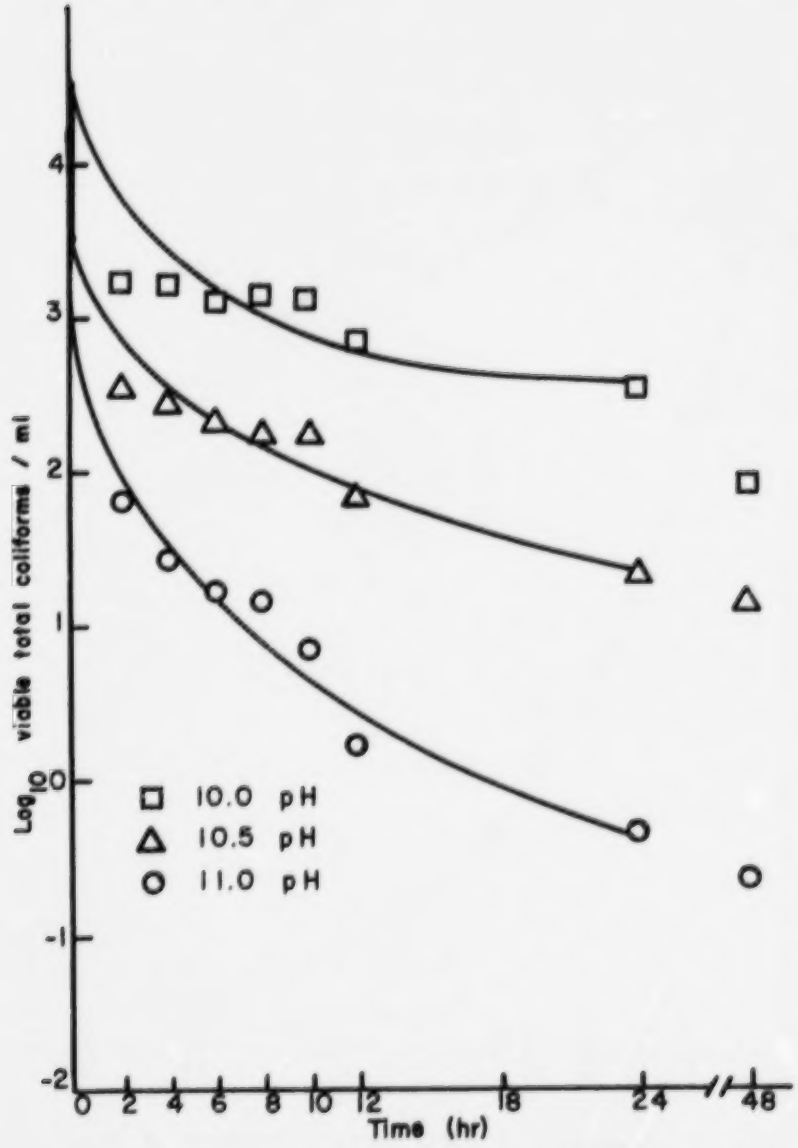

FIG. 10. Reduction of total coliform bacteria in lime treated secondary effluent at initial pH's of 10.0, 10.5 and 11.0, at 1° C.

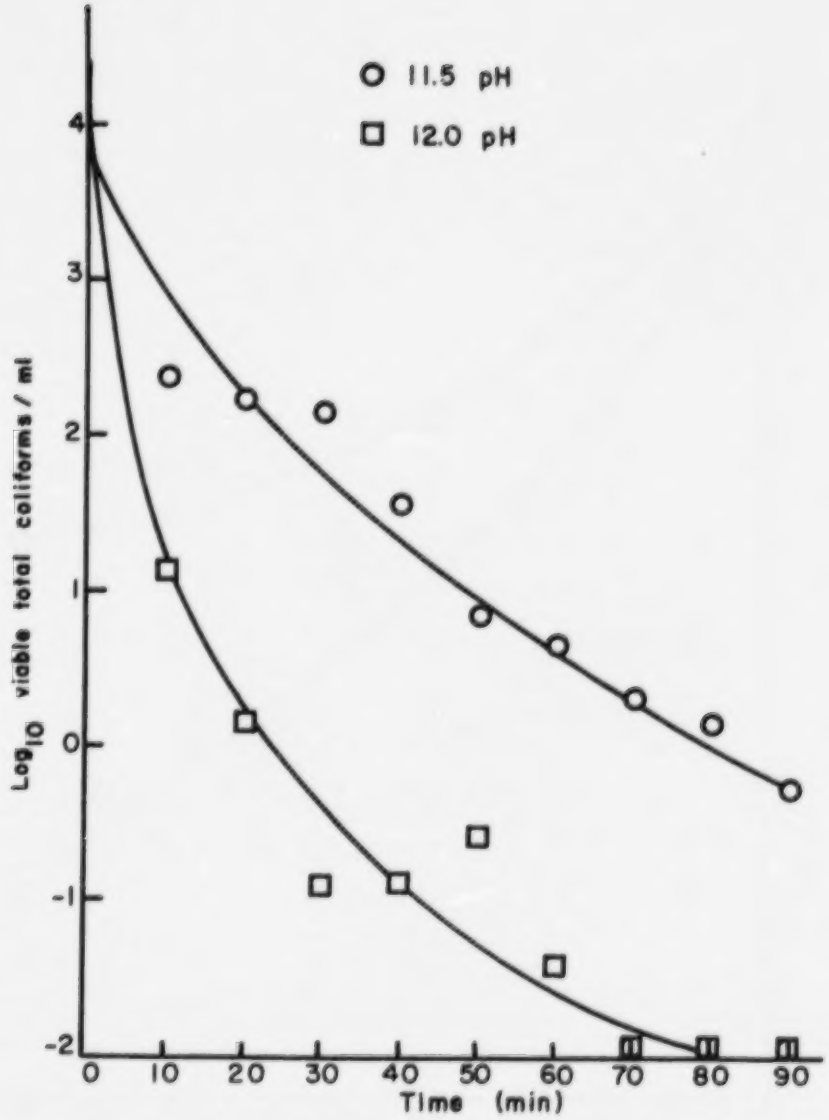

FIG. 11. Reduction of total coliform bacteria in lime treated secondary effluent at initial pH's of 11.5 and 12.0, at 10°C.

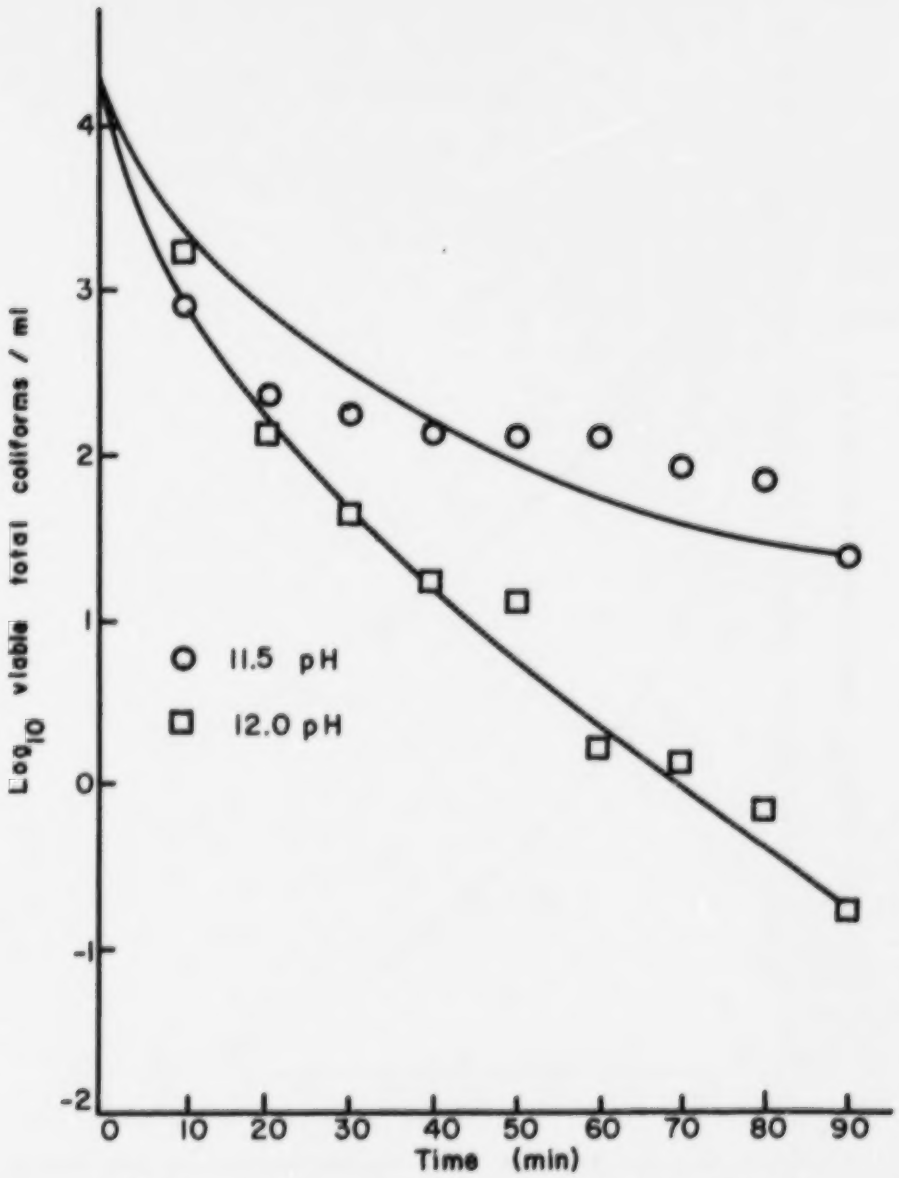

FIG. 12. Reduction of total coliform bacteria in lime treated secondary effluent at initial pH's of 11.5 and 12.0, at 1°C.

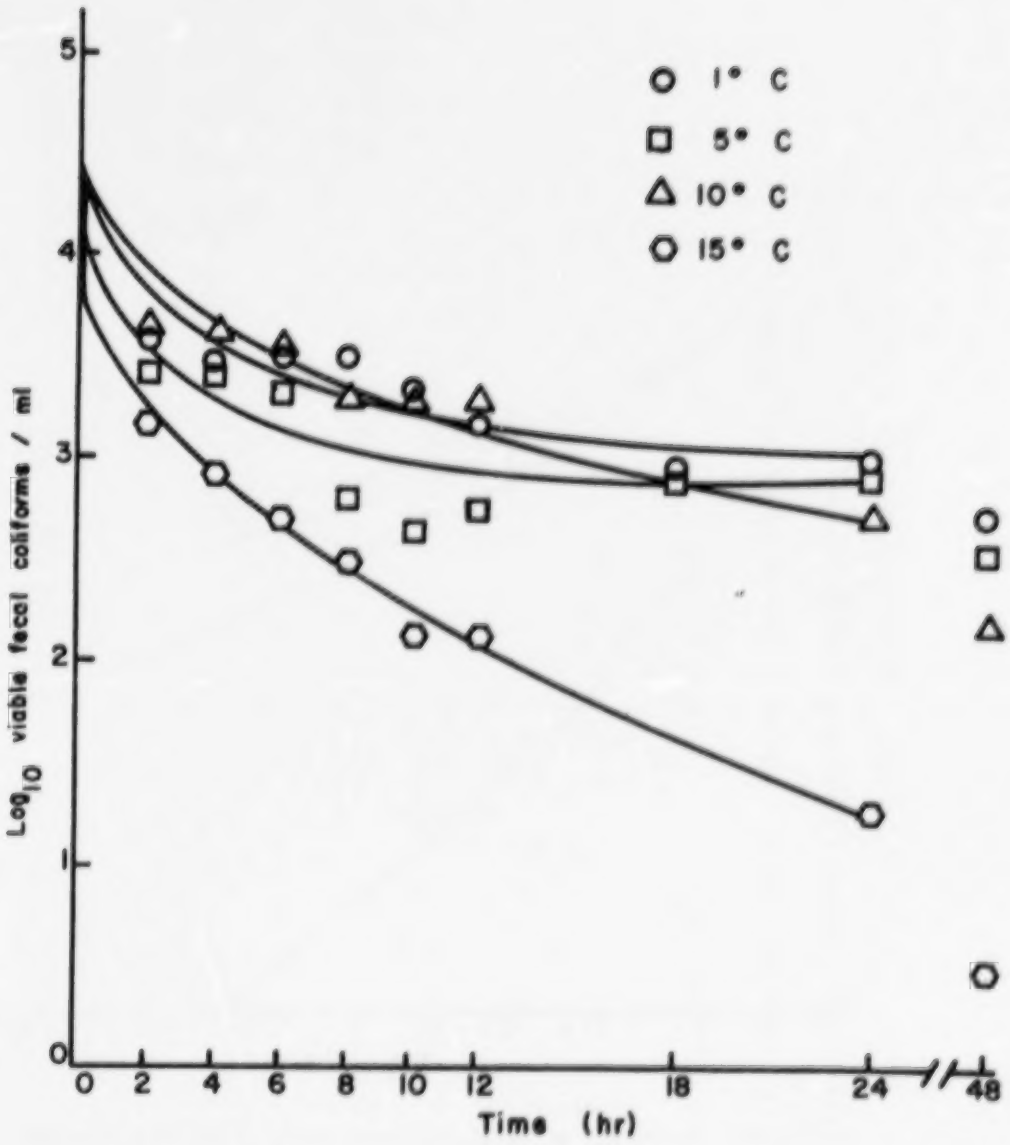

FIG. 13. Reduction of fecal coliform bacteria in lime treated sewage at an initial pH of 10.0, at 1 to 15° C.

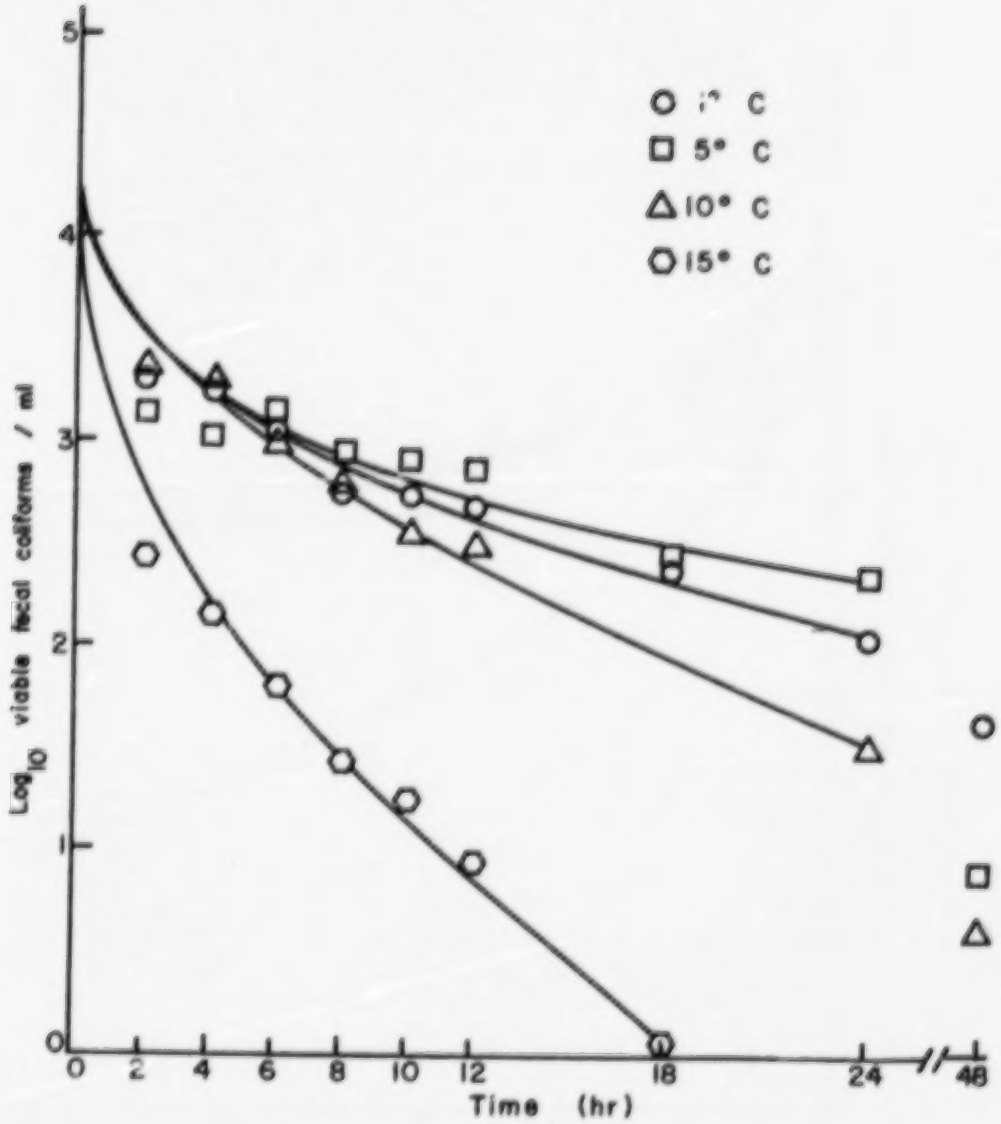

FIG. 14. Reduction of fecal coliform bacteria in lime treated sewage at an initial pH of 10.5, at 1 to 15° C.

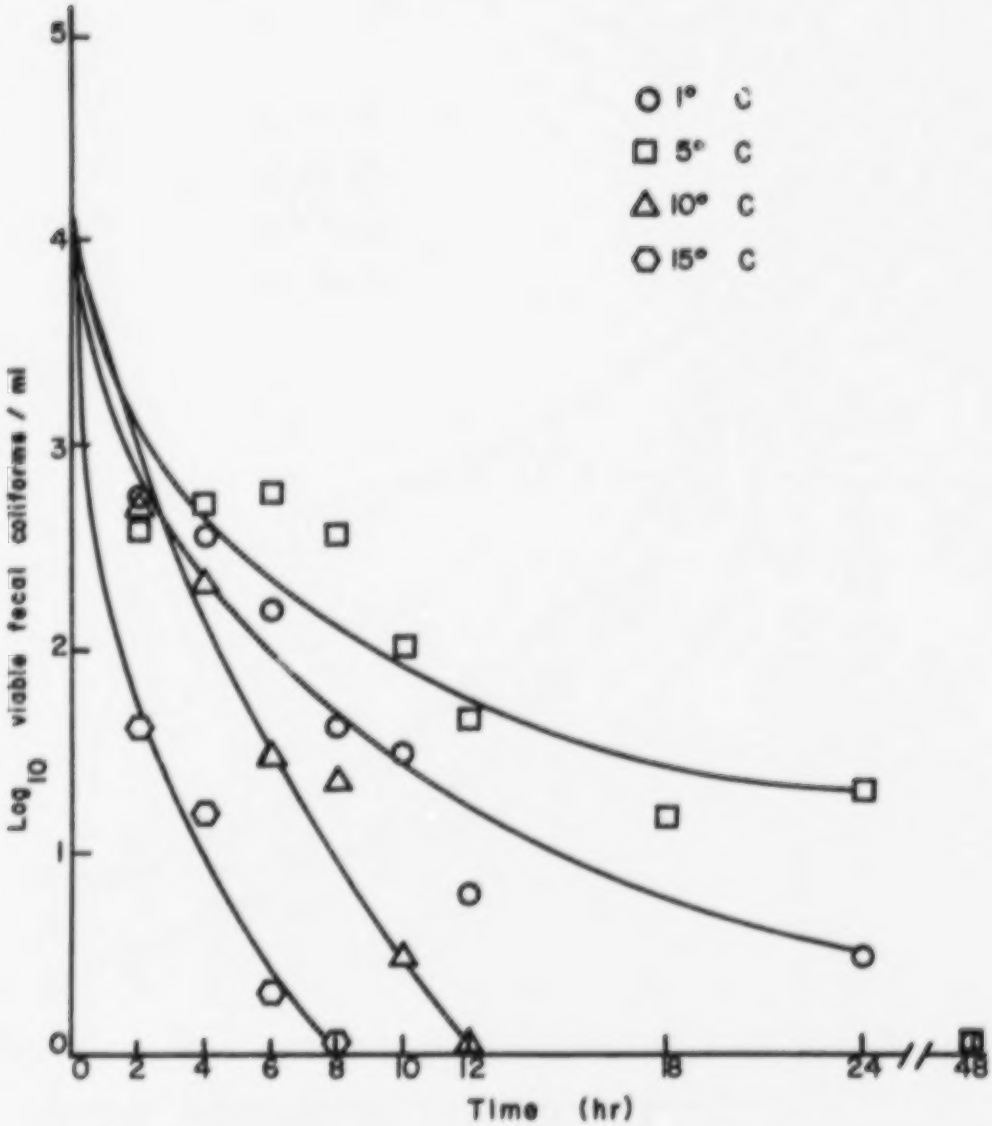

FIG. 15. Reduction of fecal coliform bacteria in lime treated sewage at an initial pH of 11.0, at 1 to 15° C.

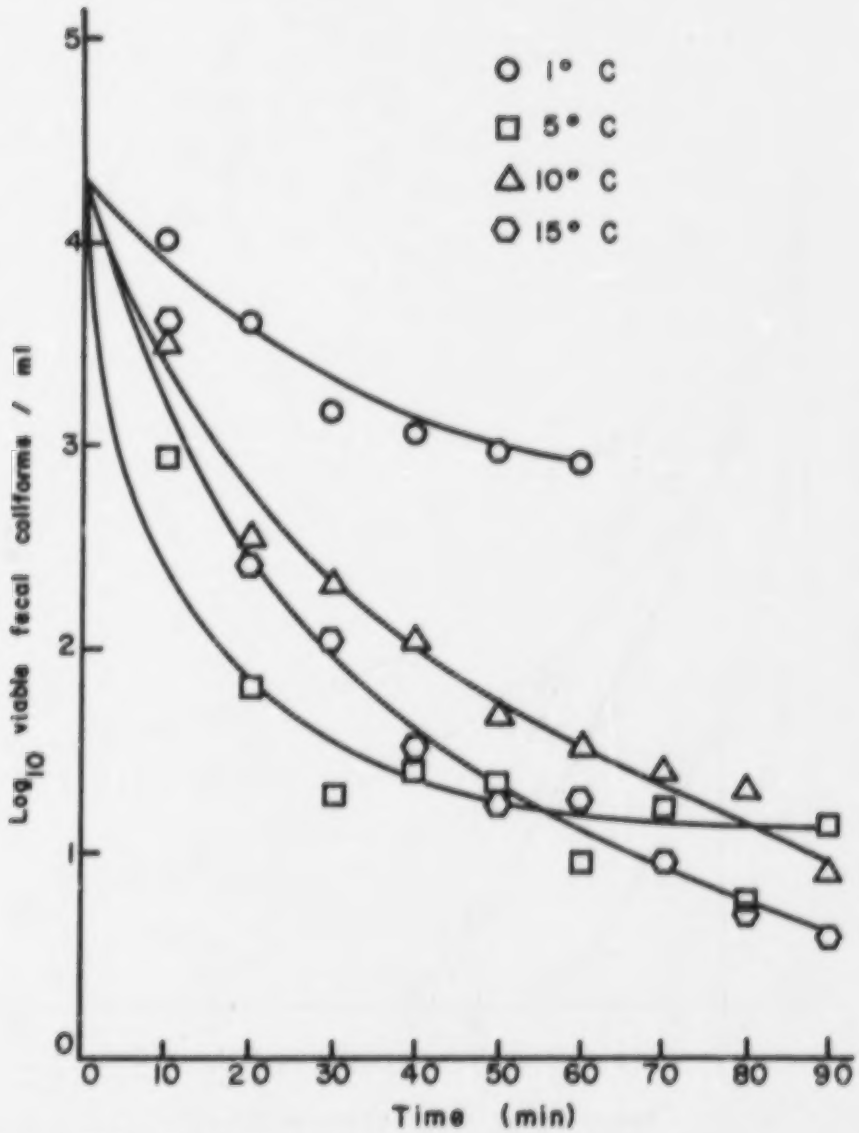

FIG. 16. Reduction of fecal coliform bacteria in lime treated sewage at an initial pH of 11.5, at 1 to 15° C.

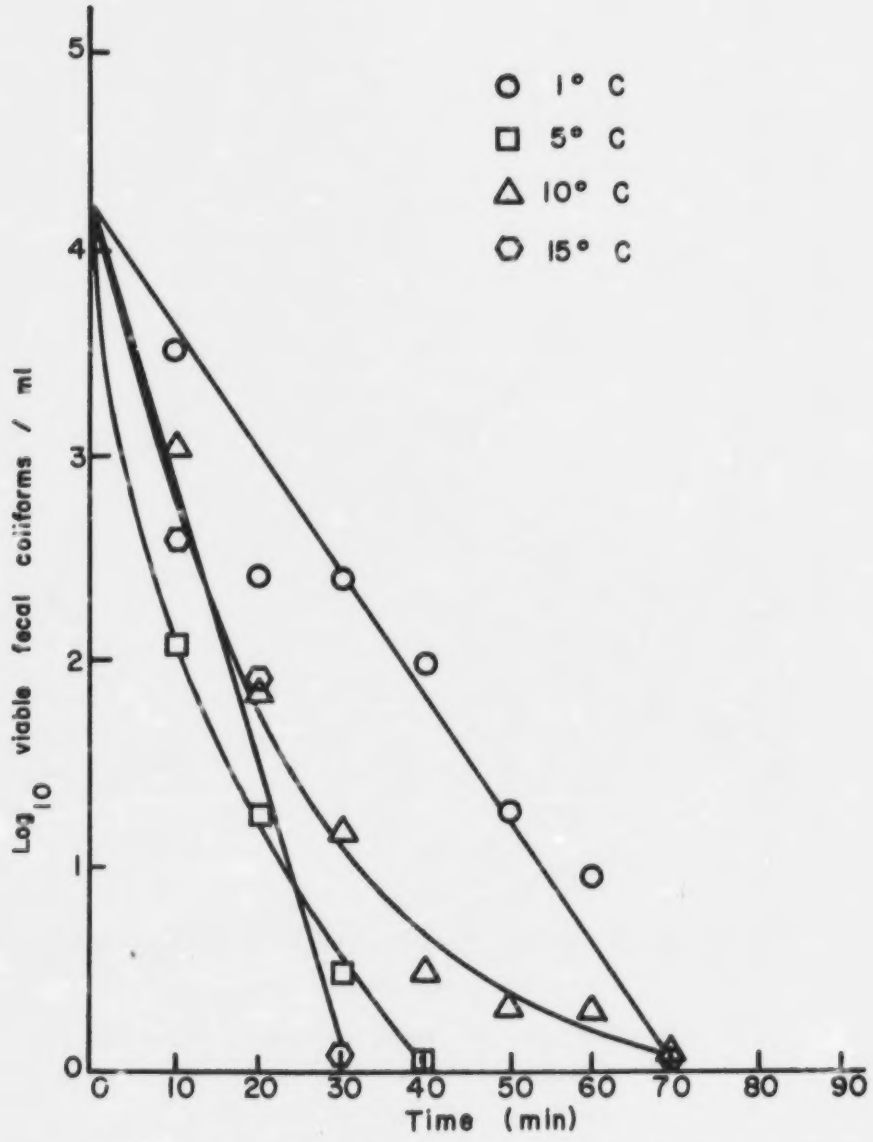

FIG. 17. Reduction of fecal coliform bacteria in lime treated sewage at an initial pH of 12.0, at 1 to 15° C.

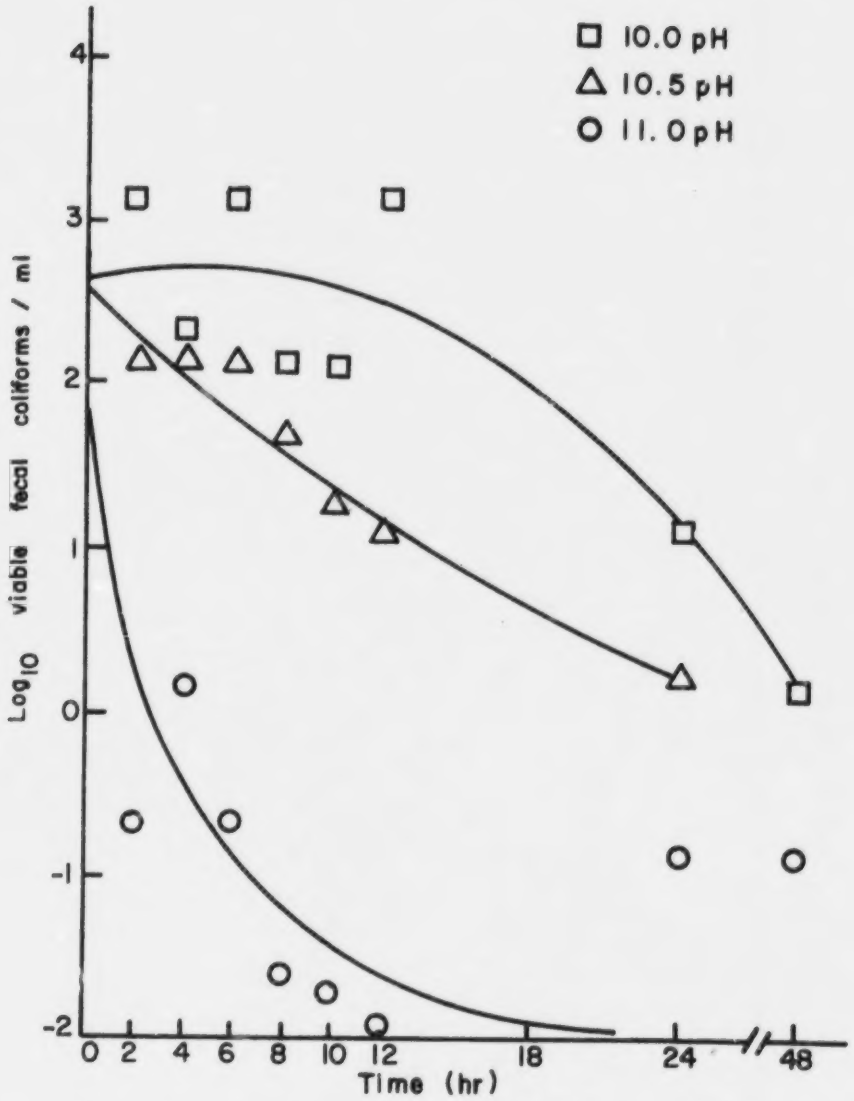

FIG. 18. Reduction of fecal coliform bacteria in lime treated secondary effluent at initial pH's of 10.0, 10.5 and 11.0, at 10° C.

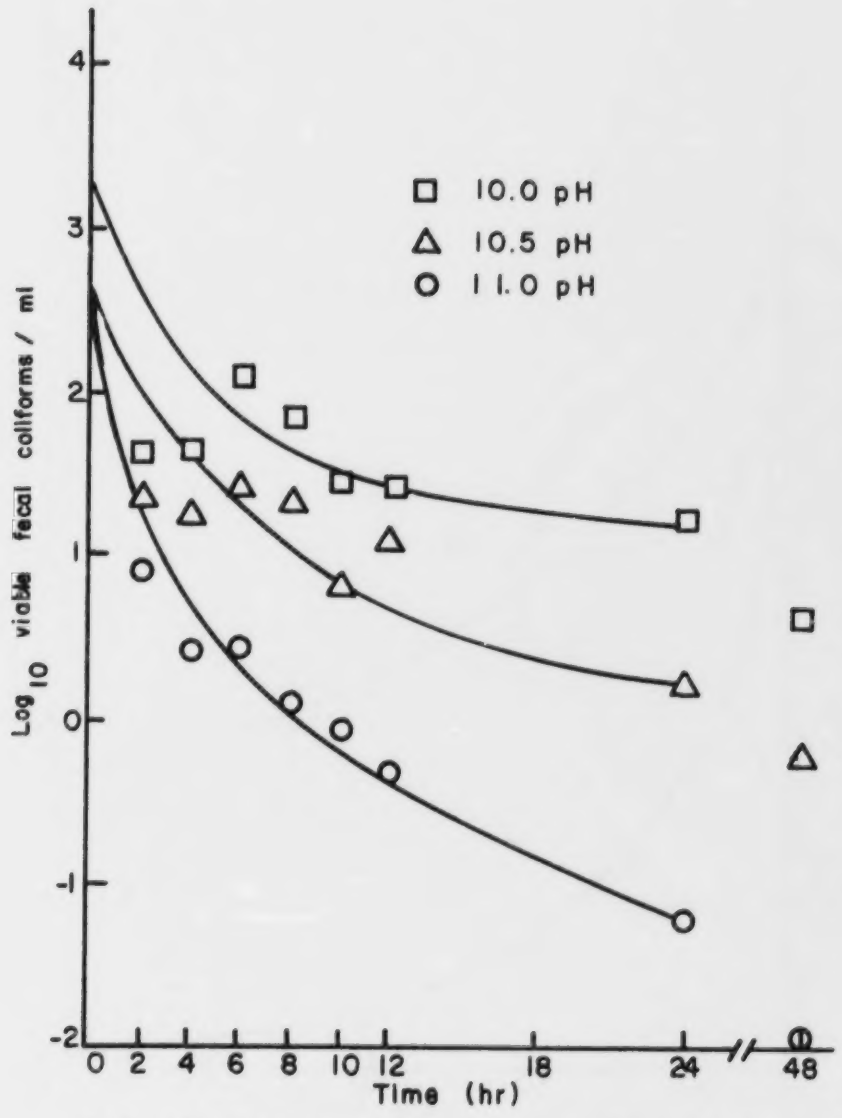

FIG. 19. Reduction of fecal coliform bacteria in lime treated secondary effluent at initial pH's of 10.0, 10.5 and 11.0, at 1° C.

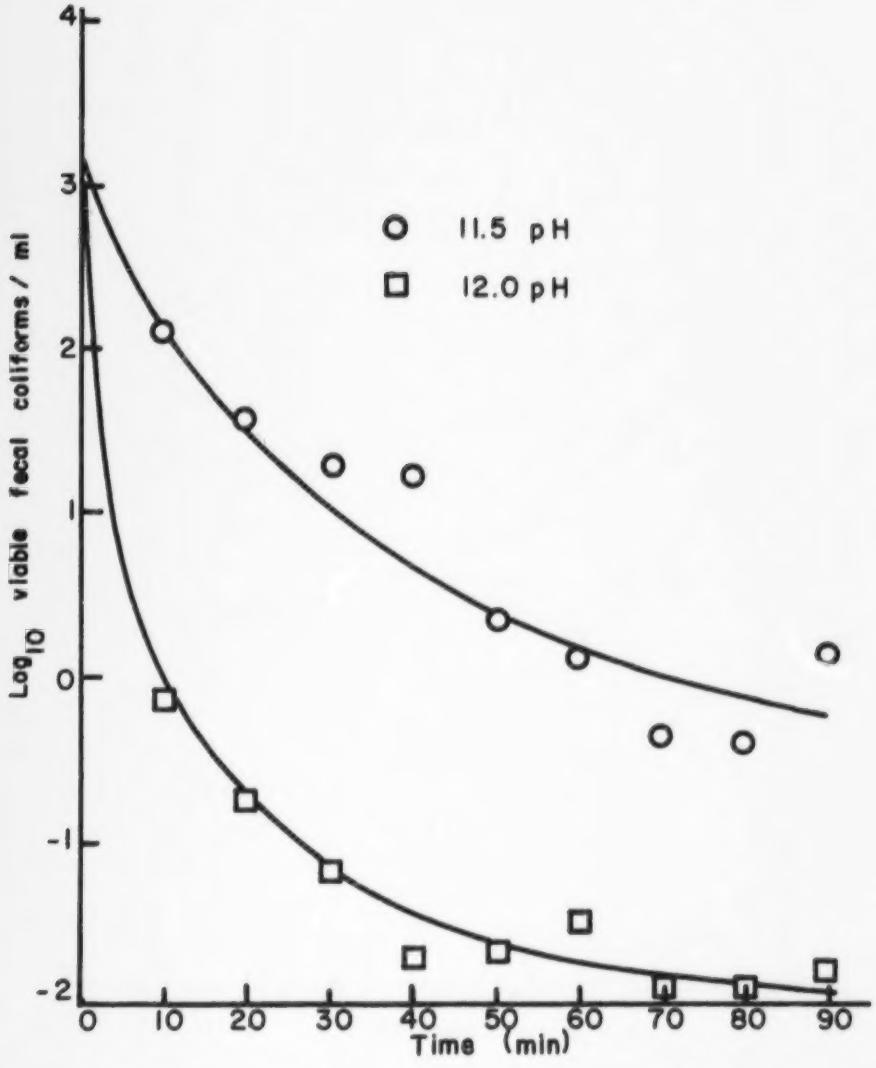

FIG. 20. Reduction of fecal coliform bacteria in lime treated secondary effluent at initial pH's of 11.5 and 12.0, at 10° C.

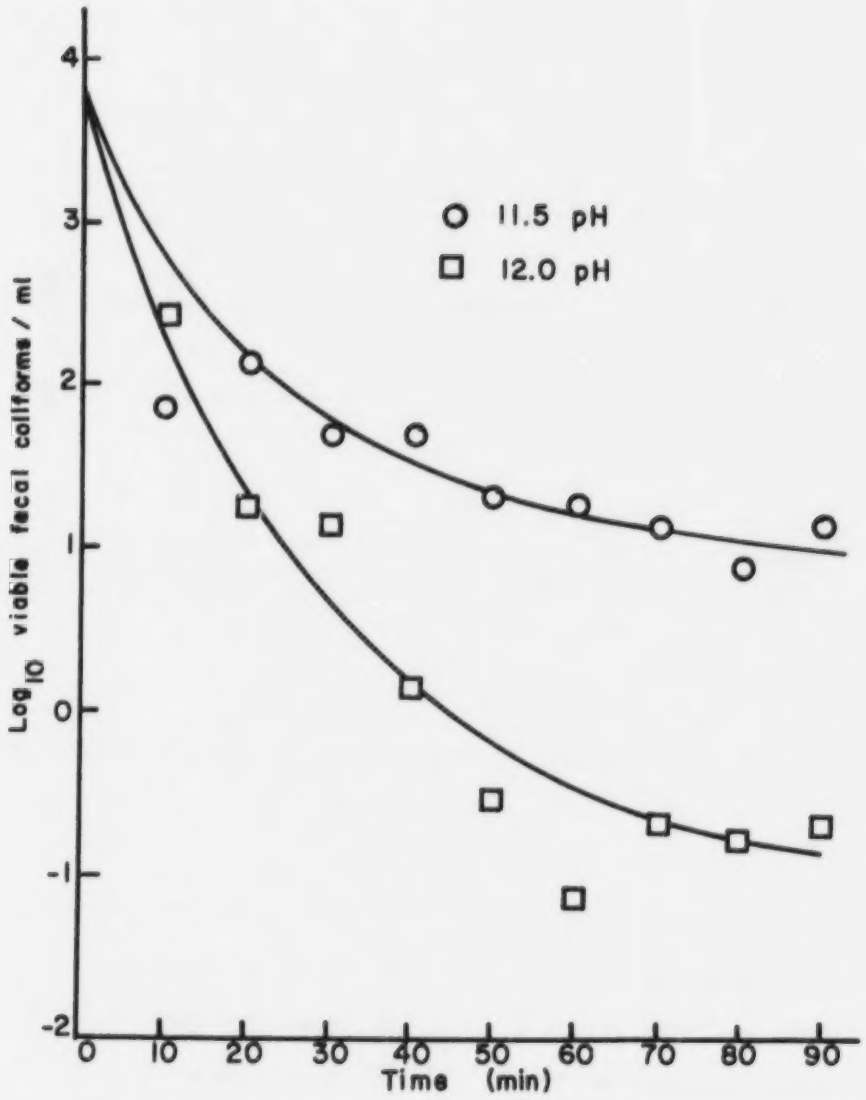

FIG. 21. Reduction of fecal coliform bacteria in lime treated secondary effluent at initial pH's of 11.5 and 12.0, at 1° C.

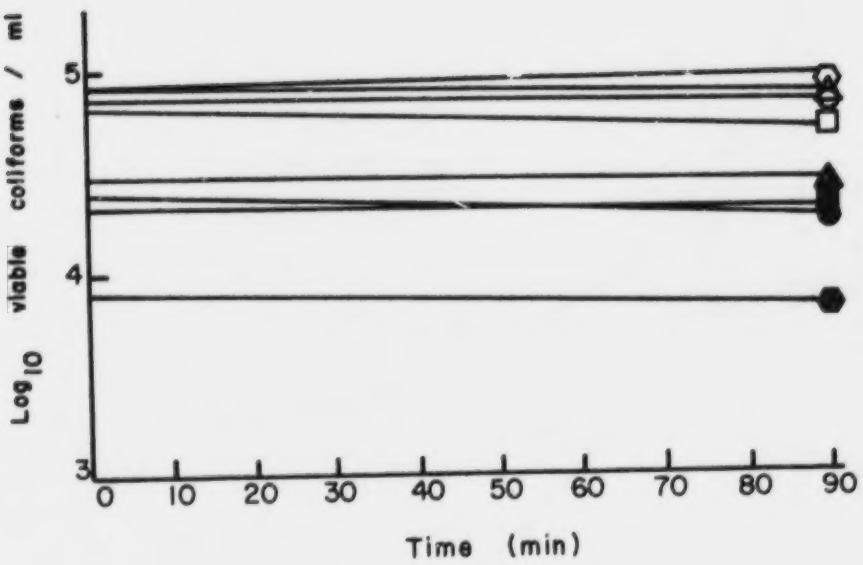

FIG. 22. Survival of total and fecal coliform bacteria in sewage controls at 1 to 15° C.

○ total coliforms at 1° C
□ total coliforms at 5° C
△ total coliforms at 10° C
⬡ total coliforms at 15° C
● fecal coliforms at 1° C
■ fecal coliforms at 5° C
▲ fecal coliforms at 10° C
⬢ fecal coliforms at 15° C

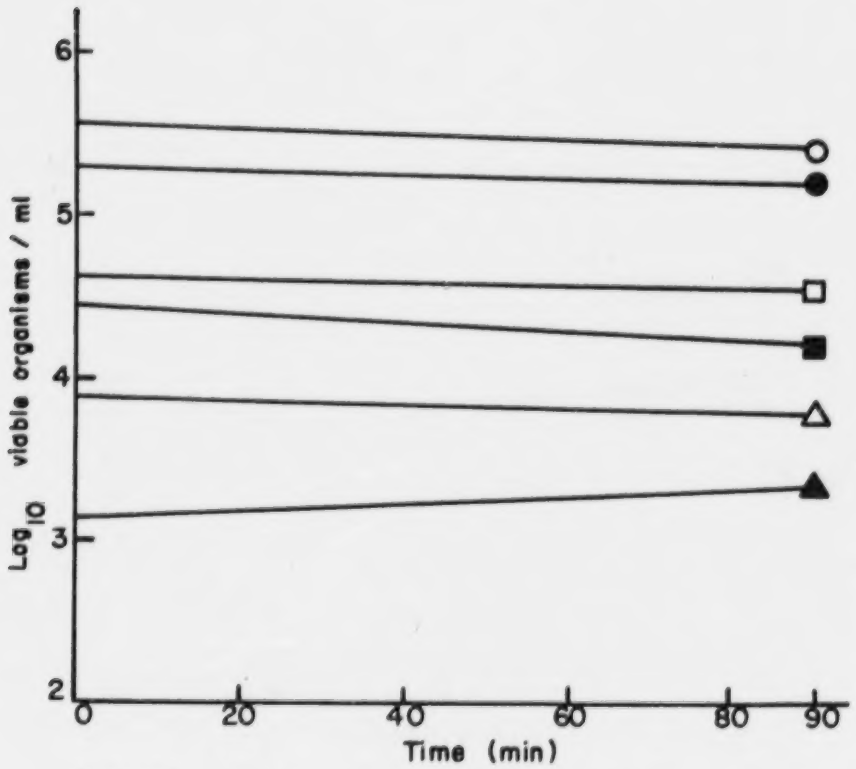

FIG. 23. Survival of bacteria in secondary effluent controls at 1 and 10°C.

□ total coliforms at 1°C ■ total coliforms at 10°C

△ fecal coliforms at 1°C ▲ fecal coliforms at 10°C

○ SPC at 1°C ● SPC at 10°C

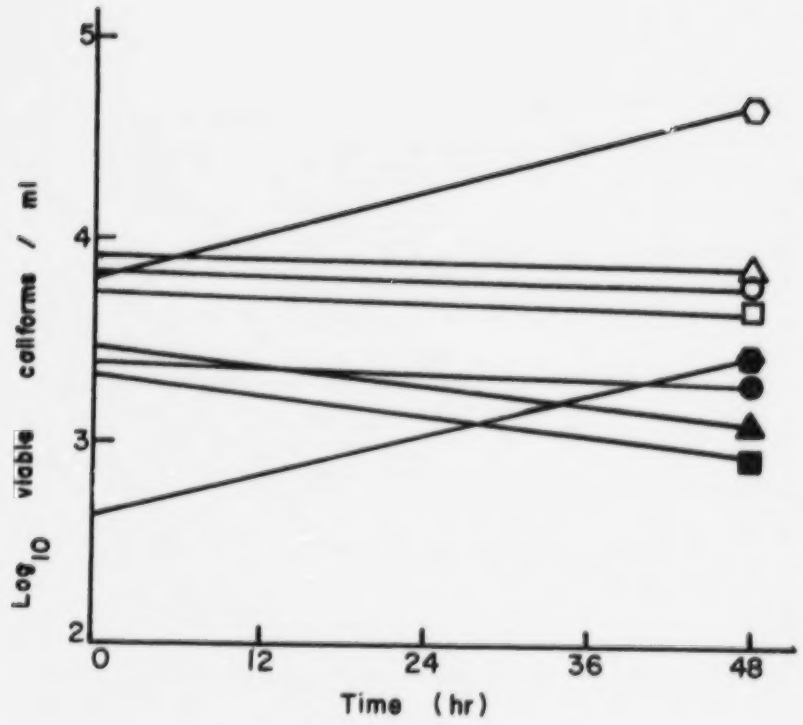

FIG. 24. Survival of total and fecal coliform bacteria in sewage controls at 1 to 15° C.

○ total coliforms at 1° C
□ total coliforms at 5° C
△ total coliforms at 10° C
⬡ total coliforms at 15° C
● fecal coliforms at 1° C
■ fecal coliforms at 5° C
▲ fecal coliforms at 10° C
⬢ fecal coliforms at 15° C

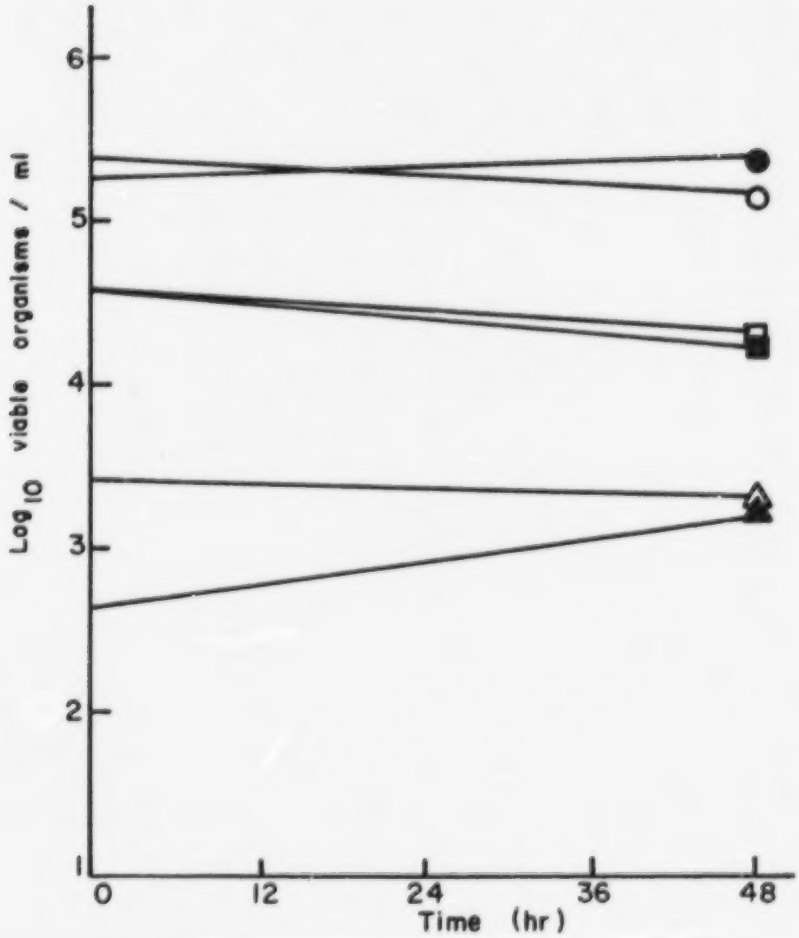

FIG. 25. Survival of bacteria in secondary effluent controls at 1 and 10° C.

□ total coliforms at 1° C
■ total coliforms at 10° C
△ fecal coliforms at 1° C
▲ fecal coliforms at 10° C
○ SPC at 1° C
● SPC at 10° C

Table 2. Survival of standard plate count organisms (SPC) in the supernatant phase of lime treated and control sewages.

Treatment temperature (C)	Sewage Type	Initial count/ml	48 hour count/ml in treatments and controls			
			pH 10.0	pH 10.5	pH 11.0	control
1	R	5.0×10^5	9,300	8,300	2,200	4.1×10^5
5	R	6.0×10^5	10,000	2,900	1,300	4.1×10^5
10	R	6.0×10^5	17,000	5,000	900	1.7×10^6
15	R	1.1×10^6	3.4×10^6	2.3×10^6	1.2×10^6	1.7×10^7
1	SE	3.4×10^5	1,000	380	110	1.8×10^5
10	SE	3.8×10^5	4.6×10^4	1.5×10^4	340	3.6×10^5

R - raw sewage
SE - secondary effluent

Table 3. Survival of standard plate count organisms (SPC) in the supernatant phase of lime treated and control sewage.

Treatment temperature (C)	Sewage type	Initial count/ml	90 minute count/ml in treatments and controls		
			pH 11.5	pH 12.0	control
1	R	7.0×10^5	15,000	1,400	6.0×10^5
5	R	5.6×10^5	5,400	620	4.8×10^5
10	R	5.8×10^5	2,400	200	5.6×10^5
15	R	8.8×10^5	1,800	200	1.0×10^6
1	SE	5.4×10^5	5,100	82	5.3×10^5
10	SE	3.7×10^5	3,000	370	3.7×10^5

R - raw sewage
SE - secondary effluent

Table 4. Comparative numbers of total coliform bacteria found in sewage supernatant and precipitated solids during lime treatment.

Treatment temperature	Initial count/ml	Exposure interval	Phase	Final counts/ml in high pH treatments				
				pH 10.0	pH 10.5	pH 11.0	pH 11.5	pH 12.0
1°C	7.6×10^4	48 hrs.	S	5.7×10^4	1.1×10^3	1.2×10^2		
			L	4.0×10^3	2.8×10^1	4.0×10^0		
	7.3×10^4	90 min.	S				6.0×10^3	3.0×10^0
			L				5.4×10^2	2.0×10^0
5°C	7.9×10^4	48 hrs.	S		8.5×10^{2a}	1.0×10^2		
			L		2.3×10^2	8.0×10^0		
	6.0×10^4	90 min.	S				6.5×10^2	8.0×10^{0a}
			L				2.6×10^1	1.0×10^0
10°C	8.1×10^4	48 hrs.	S	2.8×10^4	5.2×10^2	2.5×10^1		
			L	2.5×10^2	3.5×10^1	1.0×10^0		
	8.6×10^4	90 min.	S				3.4×10^3	4.0×10^0
			L				5.8×10^1	1.0×10^0
15°C	7.7×10^4	48 hrs.	S	1.1×10^5	5.0×10^2	2.7×10^1		
			L	4.3×10^3	1.1×10^1	1.0×10^0		
	8.7×10^4	90 min.	S				6.0×10^2	2.0×10^0
			L				1.0×10^1	1.0×10^0

[a] Only one value included in the average

L - supernatant liquid phase

S - semisolid material precipitated from sewage with lime

Table 5. Comparative numbers of fecal coliform bacteria found in sewage supernatant and precipitated solids during lime treatment.

Treatment temperature	Initial count/ml	Exposure interval	Phase	Final counts/ml in high pH treatments				
				pH 10.0	pH 10.5	pH 11.0	pH 11.5	pH 12.0
1°C	2.5×10^4	48 hrs.	S	5.3×10^4	6.0×10^3	1.5×10^2		
			L	5.4×10^2	4.5×10^2	1.0×10^0		
	2.5×10^4	90 min.	S				1.9×10^3	1.0×10^0
			L				3.8×10^1	2.0×10^0
5°C	2.6×10^4	48 hrs.	S		6.0×10^{0a}			
			L	3.6×10^2	8.0×10^0	1.0×10^0		
		90 min.	S					
			L				1.4×10^2	1.0×10^0
10°C	2.2×10^4	48 hrs.	S	1.4×10^3	4.5×10^1	0		
			L	1.5×10^2	4.0×10^0	1.0×10^0		
	3.0×10^4	90 min.	S				6.5×10^1	0
			L				8.0×10^0	1.0×10^0
15°C	4.2×10^3	48 hrs.	S	7.5×10^3	1.0×10^0	5.0×10^0		
			L	3.0×10^0	1.0×10^0	1.0×10^0		
	8.0×10^3	90 min.	S				2.8×10^2	0
			L				4.0×10^0	1.0×10^0

[a] Only one value included in the average

L - supernatant liquid phase

S - semisolid material precipitated from sewage with lime

Table 6. Comparative numbers of standard plate count bacteria found in sewage supernatant and precipitated solids during lime treatment.

Treatment temperature	Initial count/ml	Exposure interval	Phase	Final counts/ml in high pH treatments				
				pH 10.0	pH 10.5	pH 11.0	pH 11.5	pH 12.0
1°C	5.0×10^5	48 hrs.	S	3.1×10^{6a}	1.9×10^{3a}	3.0×10^{4a}		
			L	9.3×10^3	8.3×10^3	2.2×10^3		
	7.0×10^5	90 min.	S				1.7×10^6	1.4×10^5
			L				1.5×10^4	1.4×10^3
5°C	6.0×10^5	48 hrs.	S					
			L	1.0×10^4	2.9×10^3	1.3×10^3		
	5.6×10^5	90 min.	S				4.0×10^{6a}	4.5×10^{5a}
			L				5.4×10^3	6.2×10^2
10°C	6.0×10^5	48 hrs.	S	9.7×10^6	8.8×10^5	2.8×10^5		
			L	1.7×10^4	5.0×10^3	9.0×10^2		
	5.8×10^5	90 min.	S				3.1×10^5	1.1×10^5
			L				2.4×10^3	2.0×10^2
15°C	1.1×10^6	48 hrs.	S	6.6×10^8	2.2×10^8	2.0×10^7		
			L	3.4×10^6	2.3×10^6	1.2×10^6		
	8.8×10^5	90 min.	S				1.1×10^6	8.3×10^4
			L				1.8×10^3	2.0×10^2

[a] Only one value included in the average

L - supernatant liquid phase

S - semisolid material precipitated from sewage with lime

Table 7. Comparative numbers of total coliform bacteria found in secondary effluent supernatant and sludge during lime treatment.

Temp.	Initial count/ml	Exposure time	Phase	Final counts/ml in high pH treatments				
				pH 10.0	pH 10.5	pH 11.0	pH 11.5	pH 12.0
1°C	9.3×10^4	48 hr	S	3.8×10^4	1.5×10^3	6.6×10^1		
			L	9.1×10^1	1.1×10^1	2.0×10^{-1}		
	6.1×10^4	90 min	S				9.0×10^1	1.1×10^1
			L				4.4×10^1	4.0×10^{-1}
10°C	1.7×10^5	48 hr	S	3.6×10^2	4.2×10^2	1.1×10^1		
			L	1.6×10^1	4.6×10^0	4.9×10^{-1}		
	4.4×10^4	90 min	S				9.9×10^0	4.9×10^0
			L				7.5×10^{-1}	0×10^0

S - semisolid material precipitated from sewage with lime
L - supernatant liquid phase

Table 8. Comparative numbers of fecal coliform bacteria found in secondary effluent supernatant and sludge during lime treatment.

Temp.	Initial count/ml	Exposure time	Phase	Final counts/ml in high pH treatments				
				pH 10.0	pH 10.5	pH 11.0	pH 11.5	pH 12.0
1°C	4.1×10^3	48 hr	S	7.1×10^2	9.7×10^1	3.3×10^{-1}		
			L	6.2×10^0	5.5×10^{-1}	0×10^0		
	8.8×10^3	90 min	S				4.3×10^0	5.0×10^{-1}
			L				1.7×10^{-1}	3.3×10^{-1}
10°C	1.6×10^3	48 hr	S	3.3×10^1	1.8×10^1	1.2×10^0		
			L	1.9×10^0	2.0×10^{-1}	2.3×10^{-2}		
	1.2×10^3	90 min	S				2.4×10^1	3.6×10^0
			L				1.2×10^0	2.0×10^{-2}

S - semisolid material precipitated from sewage with lime
L - supernatant liquid phase

Table 9. Comparative numbers of standard plate count bacteria found in secondary effluent supernatant and sludge during lime treatment.

Temp.	Initial count/ml	Exposure time	Phase	Final counts/ml in high pH treatments				
				pH 10.0	pH 10.5	pH 11.0	pH 11.5	pH 12.0
1°C	3.8×10^5	48 hr	S	1.1×10^6	2.6×10^5	7.1×10^4		
			L	1.0×10^3	3.8×10^2	1.0×10^2		
	5.4×10^5	90 min	S				4.3×10^5	2.6×10^3
			L				5.1×10^3	8.2×10^1
10°C	3.8×10^5	48 hr	S	1.1×10^7	3.2×10^6	7.2×10^5		
			L	4.8×10^4	1.0×10^4	2.7×10^2		
	3.7×10^5	90 min	S				3.4×10^6	2.5×10^5
			L				2.9×10^3	3.7×10^2

S - semisolid material precipitated from sewage with lime
L - supernatant liquid phase

lime treatment of settled sewage. Table 10 shows the percentage of the BOD and orthophosphate concentrations removed during lime treatment of settled sewage, primary settling of raw sewage for one hour and that removed by extended settling of raw sewage in controls, all at low temperatures. Table 11 shows the percent removals in secondary effluent at 1° and 10°C. The relative magnitudes of the BOD and orthophosphate concentrations in raw, settled and lime treated sewages are shown in Figures 26 through 33.

MEASUREMENTS OF TEMPERATURE, pH AND QUANTITIES OF LIME ADDED TO SEWAGE

Using lime slurry, the pH of all sewage samples, except controls, was adjusted as close as possible to the desired treatment value. After the initial pH adjustment, the samples were allowed to stand quiescently for either a short period (90 minutes) or for an extended contact of 48 hours. The pH did not remain at the initial value but slowly declined to a slightly lower value. The initial and final pH values of the treatment containers, at the various temperatures, are shown in Tables 12 and 13.

The treatment temperatures were carefully controlled and generally varied only about $\pm$ 0.5°C. The initial and final treatment temperatures are shown in Tables 14 and 15.

The solubility of lime increases as the temperature decreases. This fact, along with observations on the relative amounts of lime needed to produce high pH values are shown in Tables 16 and 17.

MEASUREMENTS OF THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF RAW SEWAGE SAMPLES

Sewage samples were subjected to extensive physical and chemical tests. The characteristics measured, their ranges and means, and the units of measurement used in each case are presented in Table 18.

Table 10. Reductions of BOD and orthophosphate concentrations in lime treated sewage at 1°, 5°, 10° and 15°C.

Treatment pH	Contact time (hours)	Treatment temperature (C)	Percent BOD removal	Percent $o\text{-}PO_4$ removal
10.0	24	1		47.9
	48	1	55.2	
	24	5		71.4
	48	5	72.5	
	48	10	58.0	61.9
	24	15	56.4	62.4
10.5	24	1		62.5
	48	1	59.3	
	24	5		69.2
	48	5	75.3	
	48	10	59.3	71.6
	24	15	74.0	78.5
11.0	24	1		69.5
	48	1	68.6	
	24	5		78.3
	48	5	72.5	
	48	10	57.8	81.0
	24	15	77.6	80.4
11.5	1.5	1	68.6	81.9
	1.0	5	72.2	67.2
	1.0	10	53.2	73.8
	1.0	15	58.9	88.0

Table 10. (Continued)

Treatment pH	Contact time (hours)	Treatment temperature (C)	Percent BOD removal	Percent o-PO_4 removal
12.0	1.5	1	71.2	82.6
	1.0	5	66.3	79.4
	1.0	10	52.5	93.3
	1.0	15	58.9	91.0
Settled unadjusted	1.0	1	40.6	
	1.0	5	14.9	
	1.0	5	31.1	
	1.0	10	30.0	
	1.0	10	17.1	
	1.0	15	31.1	
Control unadjusted	24	1		-16.2
	48	1	31.3	
	1.5	1	47.0	-15.5
	24	5		25.1
	48	5	27.3	
	1.0	5	22.2	2.2
	48	10	30.5	-34.6
	1.0	10	48.6	47.7
	24	15	39.5	-26.3
	1.0	15	26.0	-14.0

Table 11. Reductions of BOD and orthophosphate concentrations in lime treated secondary effluent at 1° and 10°C.

Treatment pH	Contact time	Treatment temperature (C)	Percent BOD removal	Percent $o\text{-}PO_4$ removal
10.0	1 hr	1	83	77
	24 hr	1	85	93
	48 hr	1	77	83
	1 hr	10	86	87
	24 hr	10	75	90
	48 hr	10	71	79
10.5	1 hr	1	76	85
	24 hr	1	85	90
	48 hr	1	76	89
	1 hr	10	88	88
	24 hr	10	79	92
	48 hr	10	71	90
11.0	1 hr	1	77	91
	24 hr	1	85	91
	48 hr	1	78	90
	1 hr	10	87	91
	24 hr	10	77	93
	48 hr	10	75	94
11.5	60 min	1	90	89
	90 min	1	88	93
	60 min	10	91	95
	90 min	10	94	95
12.0	60 min	1	93	97
	90 min	1	94	97
	60 min	10	90	97
	90 min	10	91	96

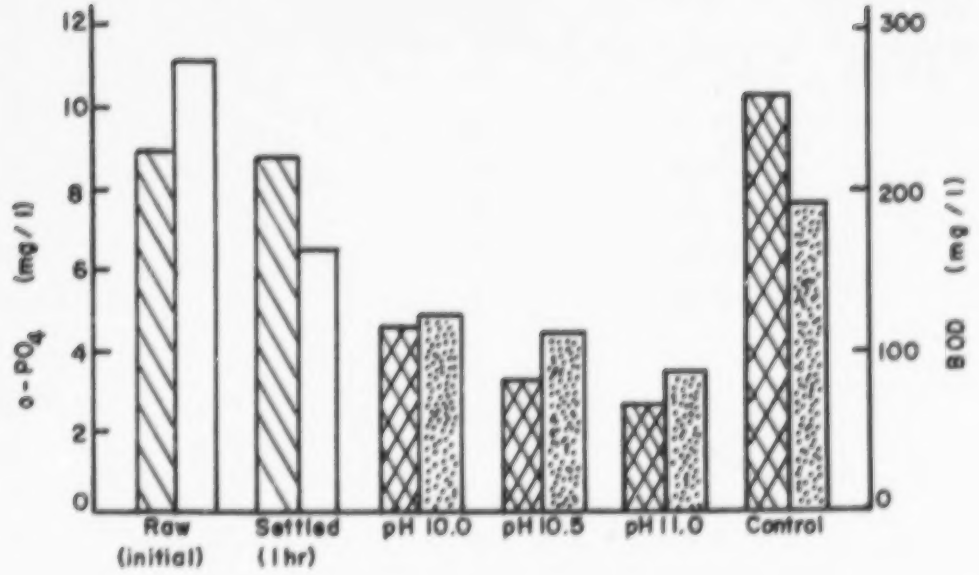

FIG. 26. Reductions in BOD and orthophosphate concentrations of sewage at pH 10.0 through 11.0, at 1°C.

Initial $o\text{-}PO_4$ concentrations

Concentration of $o\text{-}PO_4$ after 48 hours contact

Initial BOD

BOD after 24 hours contact

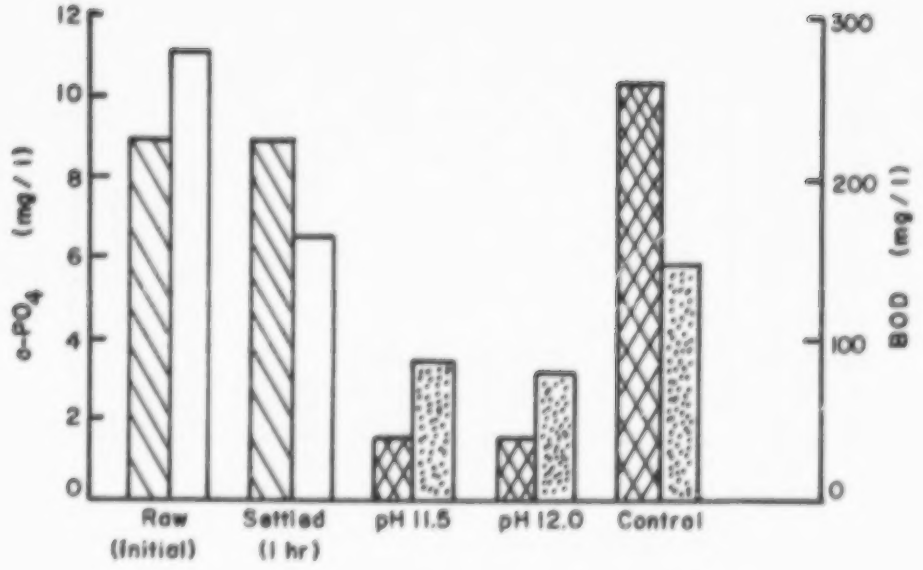

FIG. 27. Reductions in BOD and orthophosphate concentrations of sewage at pH 11.5 and 12.0, at 1°C.

Initial $o\text{-}PO_4$ concentrations

Concentration of $o\text{-}PO_4$ after 90 min contact

Initial BOD

BOD after 90 min contact

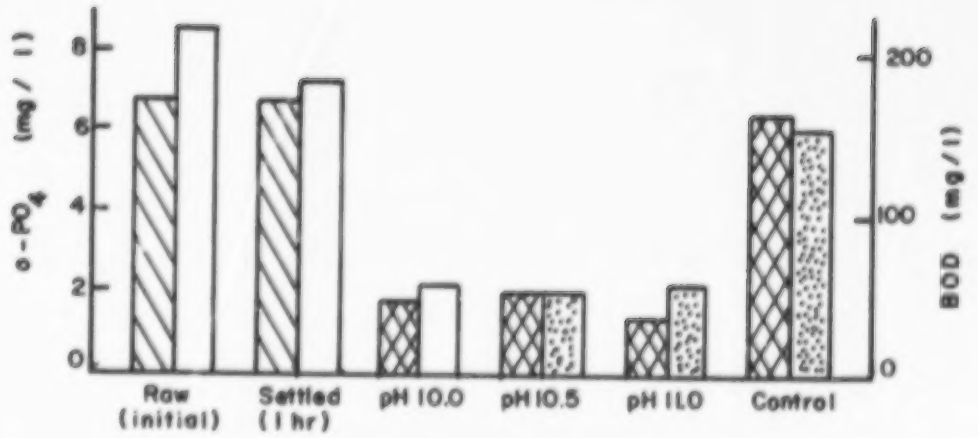

FIG. 28. Reductions in BOD and orthophosphate concentrations of sewage at pH 10.0 through 11.0, at 5° C.

Initial $o\text{-}PO_4$ concentrations

Concentration of $o\text{-}PO_4$ after 48 hours contact

Initial BOD

BOD after 24 hours contact

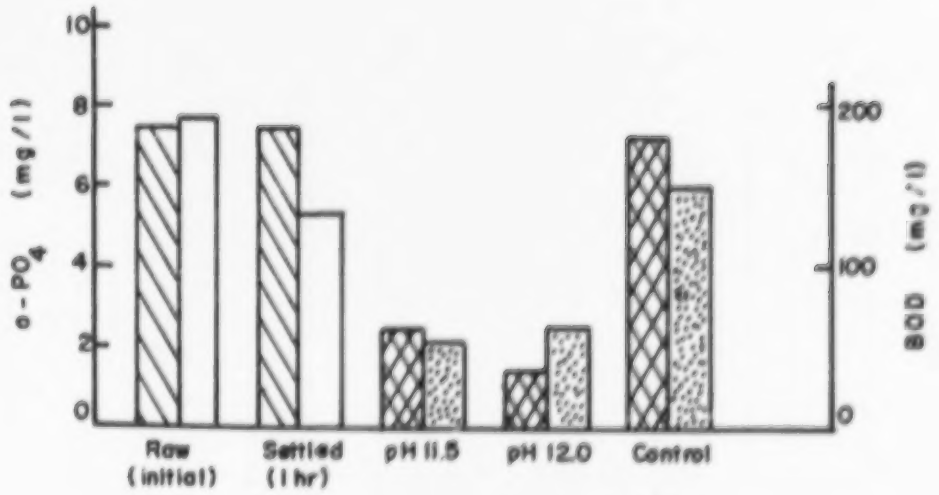

FIG. 29. Reductions in BOD and orthophosphate concentrations of sewage at pH 11.5 and 12.0, at 5° C.

Initial $o\text{-}PO_4$ concentrations

Concentration of $o\text{-}PO_4$ after 60 min contact

Initial BOD

BOD after 60 min contact

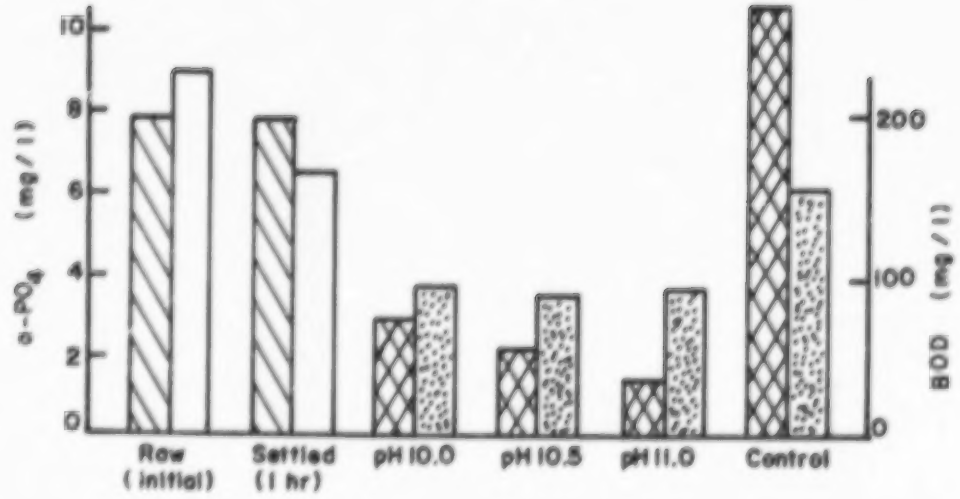

FIG. 30. Reductions in BOD and orthophosphate concentrations of sewage at pH 10.0 through 11.0, lime treatment at 10° C.

Initial $o\text{-}PO_4$ concentrations

Concentration of $o\text{-}PO_4$ after 48 hours contact

Initial BOD

BOD after 48 hours contact

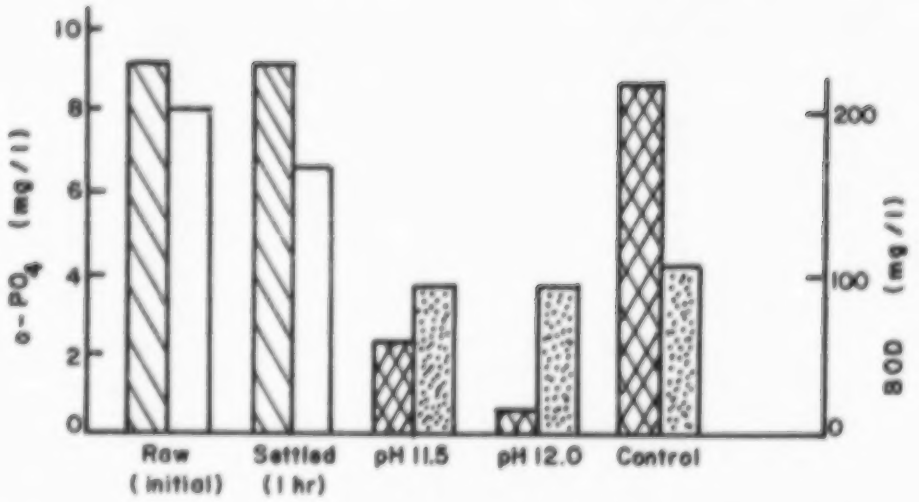

FIG. 31. Reductions in BOD and orthophosphate concentrations of sewage at pH 11.5 and 12.0, lime treatment at 10° C.

Initial $o\text{-}PO_4$ concentration

Concentration of $o\text{-}PO_4$ after 60 min contact

Initial BOD

BOD after 60 min contact

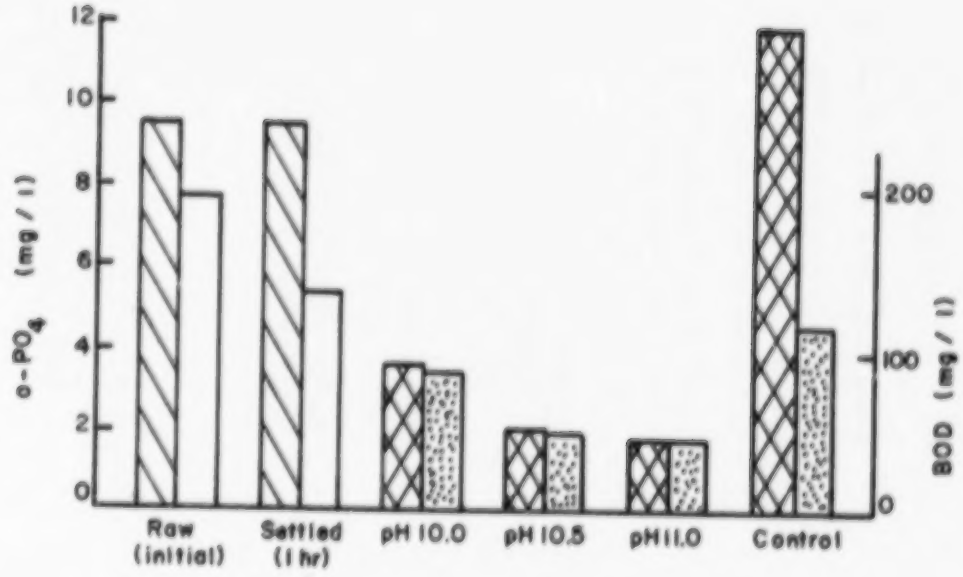

FIG. 32. Reductions in BOD and orthophosphate concentrations of sewage at pH 10.0 through 11.0, lime treatment at 15° C.

Initial $o\text{-}PO_4$ concentration

Concentration of $o\text{-}PO_4$ after 24 hours contact

Initial BOD

BOD after 24 hours contact

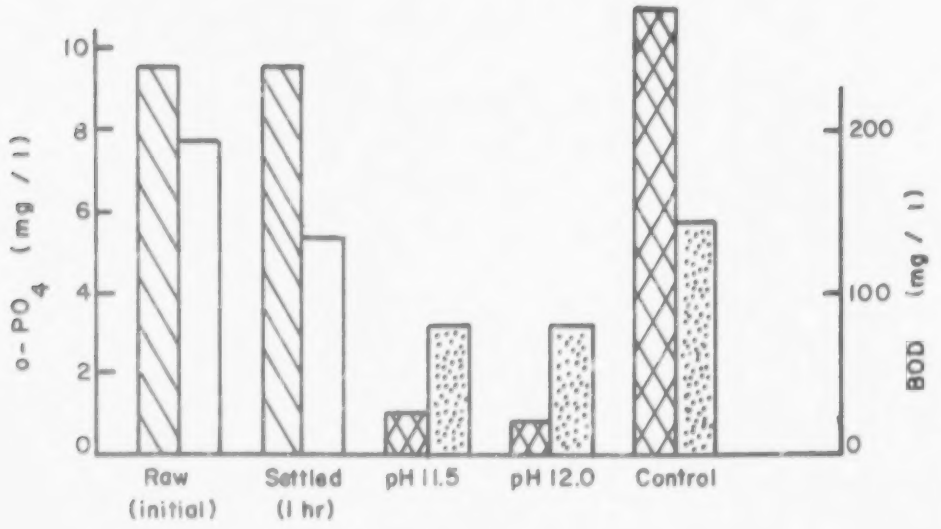

FIG. 33. Reductions in BOD and orthophosphate concentrations of sewage of pH 11.5 and 12.0, lime treatment at 15°C.

Initial $o\text{-}PO_4$ concentration

Concentration of $o\text{-}PO_4$ after 60 min contact

Initial BOD

BOD after 60 min contact

Table 12. Observed pH changes in lime treated sewage samples over the contact period.

Treatment temperature (C)	pH values									
	48 hour concact time						90 minute contact time			
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1°	10.0	9.6	10.5	10.2	11.0	10.8	11.5	11.3	12.0	11.9
5°	10.0	9.6	10.5	9.9	11.0	10.1	11.5	11.4	12.0	12.0
10°	10.0	9.8	10.5	10.0	11.0	10.5	11.5	11.4	12.0	11.9
15°	10.0	9.5	10.5	9.9	11.0	10.1	11.5	11.4	12.0	11.9

Table 13. Observed pH changes in lime treated secondary effluent over the contact period.

Treatment temperature (C)	pH values									
	48 hour contact time						90 minute contact time			
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1°	10.0	9.6	10.5	10.0	11.0	10.5	11.5	11.2	12.0	11.8
10°	10.0	9.8	10.5	9.9	11.0	10.4	11.5	11.4	12.0	11.9

Table 14. Observed temperature variations in lime treated raw sewage samples over the contact period.

Treatment temperature (C)	Measured temperatures (C)			
	48 hour contact time		90 minute contact time	
	Initial	Final	Initial	Final
1°	1.1	1.1	1.2	1.2
5°	4.7	4.9	5.2	5.3
10°	9.6	9.7	9.9	9.9
15°	15.3	15.0	15.0	14.8

Table 15. Observed temperature variations in lime treated secondary effluent over the contact period.

Treatment temperature (C)	Measured temperatures (C)			
	48 hour contact time		90 minute contact time	
	Initial	Final	Initial	Final
1°	1.0	1.2	1.3	0.9
10°	9.8	10.1	10.1	9.7

Table 16. Average quantities (ml) of lime slurry (132 mg CaO/ml) used to produce treatment pH values in 2 liter raw sewage samples.

Treatment temperature (C)	Treatment pH				
	10.0	10.5	11.0	11.5	12.0
1°	1.7	2.4	2.9	4.0	5.1
5°	1.8	2.4	2.8	3.9	6.6
10°	1.9	2.8	3.4	4.2	5.2
15°	2.0	2.8	3.1	4.2	6.3

Table 17. Average quantities (ml) of lime slurry (117 mg CaO/ml) used to produce treatment pH values in 2 liter secondary effluent samples.

Treatment temperature (C)	Treatment pH				
	10.0	10.5	11.0	11.5	12.0
1°	3.3	4.3	5.3	7.3	11.3
10°	4.8	6.7	8.3	10.0	17.0

Table 18. Ranges and means of the measured values of the various sewage characteristics (5/3/71 to 12/2/71).

Characteristic measured	Range		Mean	Units
	Low	High		
Air temperature	-2.0	25.0	16.6	C.
Sample temperature	13.9	19.0	15.9	C.
pH (20°C)	6.80	7.80	7.27	
Turbidity[1]	0.044	1.950	0.219	O.D.
Total alkalinity	200	417	242	mg/l
Settleable solids	2.7	35.0	6.35	ml/l
Suspended solids	7.1	60.0	25.1	mg/l
Dissolved solids[2]	450	1440	756	mg/l
Orthophosphate*	5.5	12.38	7.92	mg/l
Dissolved oxygen	0	1.85	0.83	mg/l
Raw BOD[3]	90	268	168	mg/l
Calcium hardness	103	180	128	mg/l
Standard plate count	3.9×10^5	1.5×10^6	9.0×10^5	per ml
Total coliforms	1.5×10^4	1.2×10^5	7.0×10^4	per ml
Fecal coliforms	2.8×10^3	3.4×10^4	1.7×10^4	per ml

* As determined by the vanadomolybdate colorimetric technique.

(1) $\text{O.D.} = \frac{\text{Klett reading} \times 2}{1000}$

(2) As determined by conductivity measurements.

(3) Standard 5-Day, 20°C test.

SECTION VI

DISCUSSION OF RESULTS

This study of lime disinfection of sewage at low temperature was predicated upon the needs of small communities located in regions with cold climates. Low rates of biological waste decomposition at low temperature as well as economic factors prompted this study of the use of elevated pH to accomplish the destruction of potentially pathogenic bacteria. Investigations were also directed toward the effects of low temperature and elevated pH values on the flocculation-coagulation of suspended sewage solids and dissolved orthophosphate.

EVALUATION OF ALKALIES FOR THE PRODUCTION OF ELEVATED pH

Investigations into the quantities of lime slurry (five parts water: one part lime) required to produce elevated pH values in settled sewage at 5°C (Figs. 1, 2) revealed that as pH increased, proportionately greater quantities of slurry were necessary to produce equivalent pH changes. This relationship held true to a pH of about 12.4, above which no further increases in pH were possible using lime. Furthermore, pH values above 12.0 often resulted in severe calcium carbonate turbidity so that the maximum practical experimental pH was about 12.0.

Similar results were obtained at temperatures of 10° and 15°C. However, because the solubility of lime (CaO) decreases as temperature increases (32), even greater amounts of lime were required to produce equivalent pH changes at the higher temperatures.

The phenomenon of requiring proportionately greater quantities of base to produce equivalent pH increases is not unique to lime. The use of 3 N potassium hydroxide produced a titration curve (Fig. 1) similar to the one with lime. The need for proportionately greater quantities of KOH became apparent at about pH 9.5, while not until about pH 11.0 when using lime. This could be attributed to the dibasic nature of calcium hydroxide. These titration values are important because they indicate that on a weight to weight basis it requires less calcium hydroxide than potassium hydroxide to produce an equivalent pH change. The economics of operation, therefore, favors the use of lime over potassium hydroxide (or other monobasic alkalies) as a sewage disinfectant and coagulant. Potassium hydroxide was tested instead of sodium hydroxide because potassium compounds are bound and utilized in the soil more readily than sodium compounds and thereby do not contribute as significantly to salinity problems.

The possibility of using a combined system of calcium hydroxide and potassium hydroxide to produce pH values above 10.0 was investigated (Fig. 3). Although such a system functions, the practicality is questionable because of the apparent interference of KOH with the

dissociation of calcium hydroxide. The apparent interference is probably a manifestation of the common ion effect (6) where a base which is strongly ionized in aqueous solutions, such as potassium hydroxide, represses the dissociation of a weakly ionized alkali, such as calcium hydroxide, when present in the same solution. As a result, large concentrations of undissociated calcium hydroxide remain at elevated pH values, the KOH being the major contributor of hydroxyl ions.

To further test the hypothesis of a common ion effect, another strongly ionizing compound (calcium chloride) containing an ion common to calcium hydroxide was added to sewage with elevated pH produced with calcium hydroxide and also with potassium hydroxide. The common ion repression effect on pH was much greater with the $CaCl_2$ - $Ca(OH)_2$ system as compared to KOH - $Ca(OH)_2$ (Table 1).

BACTERICIDAL EFFECTS OF ELEVATED pH VALUES AND LOW TEMPERATURES ON TOTAL COLIFORM BACTERIA

Disinfection of raw sewage at pH 10.0 has little value as indicated by results from bench scale studies (Fig. 4). Total coliform bacteria in supernatant liquids and precipitated solids were very resistant to this pH at cold temperature.

Similar to pH 10.0, pH 10.5 also lacks sufficient germicidal qualities to be used as a sewage disinfectant at cold temperature (Fig. 5). Even though total coliform bacteria died rapidly at this pH during the first two to four hours, death was subsequently much slower.

Total coliforms were more susceptible to the toxic effects of pH 10.5 in the supernatant phase than in the sludge or precipitated solids phase (Table 4). Either the pH of the solids was below 10.5 or the organic material present in them provided some protective colloidal effect (38).

Unlike pH 10.0 and 10.5, pH 11.0 exhibited (Fig. 6) sufficient bactericidal power to warrant further investigations as to its use as a cold temperature sewage disinfectant. Results do indicate, however, that extended contact time is necessary to achieve satisfactory results which lowers the applicability of this pH at low temperatures. Again, a protective colloidal effect was apparent because total coliforms survived significantly better in the solids than in the liquid supernatant phase (Table 4).

Total coliform bacteria succumbed rapidly to the toxic effects of pH 11.5 (Fig. 7). Furthermore, little decline in the coliform death rate throughout the contact period (90 minutes) was observed, as was the case at the lower pH values. Destruction of total coliform bacteria at pH 11.5 appears to be about 10 to 15 times more rapid than the death rate produced at pH 11.0. It also appears that some critical pH

exists (between 11.0 and 11.5) at which the lethal power of the hydroxyl ion increases greatly. Whether this increase in germicidal efficiency is due to increased hydroxyl ion concentration alone or the added lethal effect of undissociated calcium hydroxide, as proposed by Schuyler (67) during his studies, cannot be determined from this study.

Total coliform bacteria survived slightly better in the precipitated solids than in the supernatant (Table 4). However, greatly improved coliform death rates were produced at pH 11.5 in the sludge, over those produced at pH 11.0, indicating that the pH of those solids was in the critical range of 11.0 to 11.5.

Death of total coliform bacteria at pH 12.0 was rapid (Fig. 8) and exponential in character. Many effluent samples from sewages treated to pH 12.0 for 90 minutes yielded coliform counts of zero to one per milliliter. The epidemiological safety of water has been based upon the presence of absence of coliform indicator bacteria; therefore, effluents with these low concentrations of coliforms are potentially safe for reuse. Coliform death in the solid and liquid phases was approximately equivalent (Table 4). Therefore, the sludge from lime treatment of sewage should have the same epidemiological safety as the effluents.

As a general rule, bactericidal efficiency of high pH increased as the treatment temperature was elevated from 1° to 15°C (Figs. 4-8). This effect on bactericidal efficiency was more apparent at the higher pH values (11.0 to 12.0) than at the lower pH values (10.0 and 10.5). At the lower temperatures, bacterial growth was extremely retarded, possibly due to decreased enzymatic activity and lower diffusion rates of oxygen and essential nutrients through membranes. With retarded enzymatic activities, the effects of elevated pH inactivation may not be as apparent at low temperatures as they are at higher temperatures.

Lime treatment of secondary effluent at 1° and 10°C showed the same degree of destruction of total coliforms as in the raw sewage samples (Figs. 9-12; Table 6).

BACTERICIDAL EFFECTS OF ELEVATED pH VALUES AND LOW TEMPERATURES ON FECAL COLIFORM BACTERIA

Fecal coliform bacteria were not appreciably affected by exposure to pH 10.0 at low temperature (Figs. 13, 18, 19). Similar to total coliforms, a population of fecal coliforms exhibited some initial death at the onset of treatment but continued exposure produced little significant death. The survival of fecal coliforms at pH 10.0 was much more efficient in the solids than in the supernatant liquid, again lending support to a protective colloidal effect (Tables 5, 8). It can only be concluded that pH 10.0 does not provide adequate

hydroxyl ion concentration to cause the destruction of indicator coliform bacteria.

Initial fecal coliform death at pH 10.5 was greater than that produced at pH 10.0; however, final concentrations (at 24 hours) were too large to indicate that pH 10.5 has any potential as a sewage disinfectant (Figs. 14, 18, 19).

Fecal coliforms were killed almost as well in sludge as in sewage supernatants, while total coliforms survived much better in the solids. This indicates that fecal coliforms may be more susceptible to elevated pH than total coliforms (Tables 5, 8).

The toxic effects of pH 11.0 toward fecal coliforms was considerably greater than pH 10.5 (Figs. 15, 18, 19). However, even though effluents with low concentrations of fecal coliforms were produced at pH 11.0, contact periods were too long to be of practical value.

Fecal coliforms died very rapidly when exposed to pH 11.5 (Figs. 16, 20, 21). This pH produced death rates which approached a logarithmic function, indicating that it has a great potential as a sewage disinfectant. Fecal coliform concentrations were somewhat higher after 90 minutes in the precipitated solids (Tables 5, 8) than the supernatant liquid phase, again indicating that a protective effect exists.

Destruction of fecal coliform bacteria at pH 12.0 (Figs. 17, 20, 21) was very rapid, and exponential in rate. Sewage effluents containing less than one organism per milliliter were produced in 70 minutes or less, attesting to the bactericidal efficiency of this pH. Furthermore, sludges and supernatants were generally disinfected equally well at this pH (Tables 5, 8). It can be concluded that pH 12.0 is a very efficient sewage disinfectant at low temperature.

At each test pH level, fecal coliform bacteria generally died more rapidly as the treatment temperature was elevated from 1° to 15°C. The reasons for this are probably identical to those proposed for total coliforms.

BACTERICIDAL EFFECTS OF ELEVATED pH VALUES AND LOW TEMPERATURES ON TOTAL PLATE COUNTS

Total (standard) plate counts, determined initially and at the termination of elevated pH treatments, reduced bacterial concentrations greatly during treatment at pH 10.0 and 10.5 within 24 hours. However, significant numbers of organisms survived, adding further evidence that pH levels under 10.5 do not provide adequate bactericidal activity (Table 2).

Far greater numbers of bacteria were found to survive treatment at these pH treatments in precipitated solids than in the supernatant

phase (Table 6, 9).

Viable organisms on standard plate counts were reduced even further by treatment at pH 11.0 (Table 2). The reductions, however, do not indicate a sizeable increase in bactericidal efficiency of pH 11.0 over the lower pH treatments. As with the lower pH values, there was significantly greater survival of organisms in the solids than in the supernatants (Table 6, 9).

Contact periods at pH 11.5 and 12.0 were 90 minutes instead of the 48 hours used at the lower pH values because bacterial death was greatly accelerated at these higher pH values. Rapid destruction of standard plate count organisms was achieved by exposure to pH 11.5 (90 minutes or less) (Table 3). The same degree of disinfection at the lower pH values required between 12 and 48 hours. This evidence again supports the observation that some critical pH exists between 11.0 and 11.5 for bacterial death. Similarly, pH 12.0 gave rapid destruction of large numbers of sewage organisms at all temperatures; however, there were still considerable surviving organisms.

In the sludges, bacterial death did occur but not as efficiently as in the supernatant (Tables 6, 9).

Total plate count organisms appeared to survive pH 10.0 more efficiently as the temperature increased (Tables 2, 3, 6, 9). This result does not follow that for total and fecal coliforms. However, the plate counts represent many species of bacteria more resistant to high pH than coliforms. It is possible that increasing the temperature provided suitable conditions for the growth of some segment of these organisms in spite of the elevated pH. Elevating the temperature from 1° to 10°C at pH 10.5 did not appear to significantly affect the death of bacteria; however, at 15°C there was an increase in total bacterial concentrations over that found in the settled untreated sewage. Therefore, pH 10.5 has little lasting effect on total bacterial populations when the temperature is high enough to allow growth to occur.

Increasing the temperature from 1° to 10°C at pH 11.0 caused gradual reductions in total bacterial survival efficiency. At 15°C, however, growth of the initial population occurred, indicating the pH had decreased during the contact period (Tables 11, 12). The final measured pH at 48 hours was 10.1; further evidence that pH values below 11 have little value for sewage disinfection.

Survival efficiency of bacteria decreased at pH 11.5 and 12.0 as the temperature was elevated from 1° to 15°C (Tables 3, 6, 9). Both phases of sewage showed this type of temperature relationship. This evidence therefore further supports the observations that pH 11.5 and 12.0 possess effective germicidal powers.

SEWAGE CONTROLS

Untreated sewage controls were sampled at the beginning and end of each treatment series to detect bacterial death produced by factors other than pH modifications. There were slight decreases in total and fecal coliform concentrations in these controls; however, they do not appear to be related to treatment temperature because the magnitude of the reductions was approximately equal at all temperatures (Figures 22-25). Fecal coliform bacteria appear to be slightly more susceptible than total coliforms to storage at cold temperatures. It can be concluded that no significant coliform death resulted from the cold temperatures employed during this study.

OTHER FACTORS POSSIBLY INFLUENCING DISINFECTION WITH LIME

Effects of factors such as varying concentrations of organic matter, variations in the size and age of bacterial populations, inorganic constituents associated with osmotic pressure and ionic strength and their relationship to bacterial survival, possible pH activation of toxic agents present in sewage, and excretion of neutralizing material by bacteria are difficult to evaluate from the results of this study. This study does not provide information on the effects of elevated pH on the death of viruses.

Sewage contains bacteria in all stages of growth and development. Consequently, when using sewage, it is impossible to selectively compare the relative susceptibility of bacteria of one physiological age with those of another. It is possible that the initial rapid decreases in total and fecal coliform concentrations represented the death of more susceptible organisms such as those in the logarithmic stage of growth. It is also possible that these reductions also represent the death of more susceptible members of the total and fecal coliform populations. Similarly, stress factors of many types previously experienced by the organisms would condition their response to pH change.

Decreases in treatment pH values during extended contact may be the result of absorption of atmospheric and respiratory carbon dioxide along with the excretion of base neutralizing substances.

Coliform bacteria were observed to be resistant to pH values in the range of 10 to 11. It is possible that this resistance is a natural phenomenon because the normal habitat of these bacteria, the intestinal tract, has a moderately alkaline pH of 9.0 to 10.0 or possibly higher (30).

EFFECTS OF LIME TREATMENT ON THE CONCENTRATIONS OF BIODEGRADABLE ORGANIC MATTER AND ORTHOPHOSPHATE IN DOMESTIC SEWAGE

Lime treatment of settled, raw and secondary treatment sewage resulted in the removal, by precipitation, of biodegradable organic matter and dissolved orthophosphate. Primary settling of raw domestic

sewage generally resulted in 15 to 30 percent reductions in the 5-day 20°C BOD (Table 10, Figs. 26-33). Removal of large volumes of settleable solids were usually accompanied by relatively large reductions in the BOD, while minimal BOD reductions usually accompanied smaller volumes of settleable solids. Primary settling of raw domestic sewage did not produce any detectable lowering of the orthophosphate concentration, probably because of its solubility (56).

It was noted during this laboratory study that visible coagulation of sewage solids began to occur at a pH of 9.6, regardless of the treatment temperature. Subsequent precipitation of the coagulated sewage material appeared to be complete in 15 to 30 minutes.

When the supernatant liquid from primary and secondary settled sewage was subjected to elevated pH treatments, significant BOD and orthophosphate reductions were observed. Increasing the pH generally resulted in greater removals of organic matter and orthophosphate; however, increasing the temperature did not necessarily yield the same result. Relatively short contact periods (60 to 90 minutes) at the higher pH values (11.5 and 12.0) produced reductions in the BOD and orthosphosphate concentrations which were equal to or better than those produced by long contact periods (24 to 48 hours) at the lower pH values (10.0 to 11.0).

In order to more accurately evalute the effects of temperature, contact time and pH on the efficiency of organic matter removal from sewage with lime, more extensive studies directed specifically at this problem would have to be performed.

Lime treated sewage supernatants contained far less biologically oxidizable organic material than either raw or secondary settled sewages. The minimum BOD reduction produced by the combined system of settling and lime treatment of raw sewage was 52%, while the maximum was 77%; with secondary effluent the removals were 71 and 94%, respectively (Tables 10, 11). Although settling did not produce any reduction in raw sewage orthophosphate concentrations, subsequent lime treatment did. Removal of orthosphosphate appeared to be slightly improved as temperatures increased. Maximum orthosphosphate removal was 95% at pH 12.0 in 60 minutes at 15°C for raw sewage (Table 10). In secondary effluent studies the maximum removal was seen at pH 12.0 in 60 minutes at 1°C (97%). The slight temperature effect seen with raw sewage was not observed with the secondary effluent samples (Table 11), indicating that orthosphosphate reduction is not temperature dependent.

It is interesting to note that sewage controls with an unmodified pH showed increases in orthophosphate concentrations over raw and settled sewage concentrations. This increase was probably due to death and lysis of a portion of the biological population, liberating orthophosphate into the medium. There is also the possibility that these in-

creases in orthophosphate concentrations were due to sudden shifts in environmental conditions such as the sudden rapid cooling to which the sewage samples were subjected to attain treatment temperatures. Mirrett (49) noted that it has been shown that bacteria release a portion of their "luxury" phosphate when the dissolved oxygen concentration is suddenly reduced. A similar mechanism may have occurred when sewage temperatures were rapidly lowered causing stress conditions with the release of "luxury" phosphate.

This study of cold temperature lime treatment of raw domestic sewage produced effluents containing concentrations of biologically oxidizable materials as low as 46.3 mg/l, and orthophosphate concentrations as low as 0.87 mg/l. When compared with raw sewages containing approximately 200 mg/l of biologically oxidizable material and 10 mg/l of orthophosphate, it can be seen that this system of sewage treatment could be a valuable tool for the reduction of water nutrients.

Measurements of many chemical and physical sewage characteristics were made on samples obtained throughout this study. Mean values and ranges are shown in Table 17. Of the characteristics measured, sewage temperature, pH and bacterial concentrations exhibited the least variation over the eight month sampling period. It was fortunate that bacterial concentrations remained fairly uniform because this allowed more reliable comparisons of the rates of coliform disinfection from different samples. The effects of the wide variation of some of the sewage characteristics on the disinfection of sewage bacteria cannot be evaluated.

SUMMARY

In this study of lime disinfection of settled domestic sewages at low temperature, it was shown that rapid destruction of coliform indicator bacteria occurred at pH 11.5 and 12.0, even at 1°C. Total and fecal coliform concentrations in sewage effluents and precipitated solids were reduced to about 10/ml (1000/100 ml) with a 90 minute contact period. Treatment at pH values at or below 11.0 failed to adequately disinfect effluents within a reasonable time period at any of the treatment temperatures studied.

Results showed that the rate of coliform disinfection at pH 11.5 and 12.0 was greatly increased over that produced at the lower pH values studied. Indications are that some critical factor exists which influences the rate of disinfection at these higher pH values. Whether this factor is pH alone, or a combination of pH, osmotic pressure and some threshold phenomenon, however, cannot be determined from these studies.

These laboratory studies indicate that a system of lime disinfection may be applicable to small sewage treatment system in regions of the world, such as Alaska, where severely cold climatic conditions prevail throughout much of the year.

In conjunction with disinfection, high pH systems also produce large reductions in the concentration of biologically oxidizable organic materials from settled sewage. A combined system of primary settling and lime treatment, at pH 12.0, produced a 71% reduction in the BOD in 90 minutes at 1°C; with secondary effluent the comparable removal was 94%.

A valuable advantage to the treatment of sewage with lime over conventional systems now in use is the large reductions in orthophosphate concentrations obtained with lime, even at very low temperatures (1° to 15°C).

SECTION VII

REFERENCES

1. Albertson, O. E., and R. J. Sherwood. Phosphate Extraction Process. J. Water Pollut. Contr. Fed. 41:1467-1490, 1969.

2. American Public Health Association. Standard Methods for the Examination of Water and Wastewater, 13th ed. New York, American Public Health Association, Inc., 1971. 874 p.

3. Anonymous. Tests of Lime-electrolytic-agitation Sewage Treatment Processes at Easton, Pennsylvania. Eng. News Rec. 83:569-573, 1919.

4. Bahlman, C. Bactericidal Action of Lime in Subcaustic Doses. Ohio Conf. Water Purif., 8th Annu. Rep., 1928.

5. Benzie, W. J., H. O. Larkin, and A. F. Moore. Effects of Climatic and Loading Factors on Trickling Filter Performance. J. Water Pollut. Contr. Fed. 35:445-455, 1963.

6. Blaedel, W. J., and V. W. Meloche. Elementary Quantitative Analysis. Theory and Practice, 2nd. ed. New York, Harper and Row, 1963. 964 p.

7. Boyd, W. L., and J. W. Boyd. Water Supply and Sewage Disposal Developments in the Far North. J. Amer. Water Works Ass. 57:858-868, 1965.

8. Boyd, W. L., and J. W. Boyd. Microbiological Studies of Aquatic Habitats of the Area of Inuvik, Northwest Territories. Arctic. 20: 27-41, 1967.

9. Burrows, W. Textbook of Microbiology, 19th ed. Philadelphia, W. B. Saunders Co., 1968. 974 p.

10. Butterfield, C. T. Bactericidal Properties of Chloramines and Free Chlorine in Water. Pub. Health Rep., 63, 934, 1948. Cited in: Chambers, C. W. Chlorination for Control of Bacteria and Viruses in Treatment Plant Effluents. J. Water Pollut. Contr. Fed. 43:228-241, 1971.

11. Buzzell, J. C. Jr., and C. N. Sawyer. Removal of Algal Nutrients from Wastewater. J. Water Pollut. Contr. Fed. 39:R16-R24, 1967.

12. Carey, F. Old Bacteria Found in Antarctica. J. Water Pollut. Contr. Fed. 34:200, 1962.

13. Chambers, C. W., and G. Berg. Disinfection and Temperature Influences. In: International Symp. on Water Pollut. Contr. in Cold Climates. U. of Alaska, 1970. p. 312-328.

14. Churchill, J. A. Water Treatment and Examination. 1st ed. Longman Group Ltd., 1970. 513 p.

15. Clark, L. K., A. J. Alter, and C. J. Blake. Sanitary Waste Disposal for Navy Camps in Polar Regions. J. Water Pollut. Contr. Fed. 34:1219-1234, 1962.

16. Cohen, B. The Effects of Temperature and Hydrogen Ions upon the Viability of B. coli and B. typhosum in Water. J. Bacteriol. 7:183-230, 1922.

17. Cohen, B., and W. M. Clark. The Growth of Certain Bacteria in Media of Different Hydrogen Ion Concentrations. J. Bacteriol. 4:409-427, 1919.

18. Culp, R. L. The Tahoe Process for Nutrient Removal. 7th Indust. Water and Waste Conference. Texas Water Pollut. Contr. Ass., 1967. p. 1-3.

19. Dickens, H. B. Water Supply and Sewage Disposal in Permafrost Areas of Northern Canada. Polar Rec. 9:421-432, 1959.

20. Doyle, C. B. Effectiveness of High pH for Destruction of Pathogens in Raw Sludge Filter Cake. J. Water Pollut. Contr. Fed. 39:1403-1409, 1967.

21. Dryden, F. D., and G. Stern. Renovated Wastewater Creates Recreational Lake. Environ. Sci. & Technol. 2:268, 1968. Cited in: Nesbitt, J. B. Phosphorous removal - the state of the art. J. Water Pollut. Contr. Fed. 41:701-713, 1969.

22. Eddy, H. P. The Significance of "Hydrogen Ion Concentration" in Water Purification. Eng. and Contracting. 56:354-355, 1921.

23. Edwards, E. T. Combination of Excess Lime, Double Coagulation and Adjustment of pH at Ironton. Ohio Conf. Water Purif., 6th Annu. Rep., 1926.

24. Edwards, E. T. Use of Lime as a Water Purification Agent at Ironton. Ohio Conf. Water Purif., 10th Annu. Rep., 1930.

25. Eliassen, R., and G. Tchnobanoglous. Chemical Processing of Wastewater for Nutrient Removal. J. Water Pollut. Contr. Fed. 40: R171-R180, 1968.

26. Endemann, H. Proc. Amer. Chem. Soc. 1:24, 1878. Cited in: McCulloch, E. C. The Germicidal Efficiency of Sodium Hydroxide. J. Bacteriol. 25:469-493, 1933.

27. Gordon, J. E., and F. L. Babbott Jr. Acute Intestinal Disease in the Arctic. J. Pub. Health. 49:1441-1453, 1959.

28. Gordon, R. C. Winter Survival of Fecal Indicator Bacteria in a Subarctic Alaskan River. Environmental Protection Agency, Corvallis, Oregon. Publication Number EPA-R2-72-013. 1972.

29. Grabow, W. O. K., N. A. Grabow, and J. S. Burger. The Bactericidal Effect of Lime Flocculation/floatation as a Primary Unit Process in a Multiple System for the Advanced Purification of Sewage Works Effluent. Water Res. 3:943-953, 1969.

30. Grollman, S. The Human Body. Its structure and physiology, 2nd ed. London, The Macmillan Co., 1969. 543 p.

31. Hach Engineer's Laboratory Methods Manual. 7th ed. Ames, Hach Chemcial Co.

32. Handbook of Chemistry and Physics, 47th ed. Cleveland, Chemical Rubber Co. 1966.

33. Hoover, C. P. Water Softening as an Adjunct to Water Purification. J. Amer. Water Works Ass. 17:751-759, 1927.

34. Hoover, C. P., and R. D. Scott. Lime Sterilization of Water. Eng. Rec. 68:257-259, 1913.

35. Houston, A. C. Purification of Water Supplies. Eng. and Contracting. 62:821-823, 1924.

36. Keefer, C. E. Temperature and Efficiency of the Activated Sludge Process. J. Water Pollut. Contr. Fed. 34:1186-1196, 1962.

37. Kehr, R. W., and C. T. Butterfield. Notes on the Relation Between Coliforms and Enteric Pathogens. Pub. Health Rep. 58:586-607, 1943.

38. Lamanna, C., and M. F. Mallette. Basic bacteriology, 3rd ed. Baltimore, The Williams and Wilkins Co., 1965. 1001 p.

39. Lange, B. [Keimmenge und desinfektionserfolg. Ein beitrag zur methodik von desinfektionsversuchen.] Z. Hyg. Infektionskr. 96:92-117, 1922. Cited in: Watkins, J. H., and C. E. A. Winslow. Factors Determining the Rate of Mortality of Bacteria Exposed to Alkalinity and Heat. J. Bacteriol. 24:243-265, 1932.

40. Lanphear, R. S. Lime Treatment of Sewage Compared with Direct Oxidation. Eng. News Rec. 89:276-278, 1922.

41. Lecompte, A. R. Water Reclamation by Excess Lime Treatment of Effluent. Tappi. 49:121A-124A, 1966.

42. Levine, M., E. E. Peterson, and J. H. Buchanan. Germicidal Efficiency of Sodium Hydroxide and Sodium Hydroxide-carbonate Mixtures at the same H-ion Concentration. Ind. Eng. Chem. 20:63-65, 1928.

43. Levine, M., J. H. Toulouse, and J. H. Buchanan. Effect of Addition of Salts on the Germicidal Efficiency of Sodium Hydroxide. Ind. Eng. Chem. 20:179-180, 1928.

44. Ludzack, F. J., R. B. Schaffer, and M. B. Ettinger. Temperature and Feed as Variables in Activated Sludge Performance. J. Water Pollut. Contr. Fed. 33:141-156, 1961.

45. Malina, J. F., and S. Tiyaporn. Effects of Synthetic Detergents on Lime-soda Ash Treatment. J. Amer. Water Works Ass. 56:727-737, 1964.

46. McCulloch, E. C. The Germicidal Efficiency of Sodium Hydroxide. J. Bacteriol. 25:469-493, 1933.

47. McCulloch, E. C., and S. Costigan. A Comparison of the Efficiency of Phenol, Liquor Cresolis, Formaldehyde, Sodium Hypochlorite, and Sodium Hydroxide against Eberthella typhi at Various Temperatures. J. Infec. Dis. 59:281-284, 1936.

48. McKinney, R. E. Microbiology for Sanitary Engineers. New York, McGraw-Hill Book Company, Inc., 1962. 293 p.

49. Mirrett, S. Bacterial Uptake of Aquatic Phosphorus. M.S. Thesis. Colo. State Univ. Fort Collins, Colo. 1971. 111 p.

50. Mudge, C. S., and B. M. Lawler. Effects of Alkali Solutions on Bacteria Found in Unwashed Milk Bottles. Ind. Eng. Chem. 20:378-380, 1928.

51. Mulbarger, M. C., E. Grossman III, and R. B. Dean. Lime Clarification, Recovery and Reuse for Wastewater Treatment. U.S. Dept. Interior. Fed. Water Pollut. Contr. Admin. Cincinnati Water Res. Lab. Cincinnati, Ohio, 1968.

52. Myers, R. P. The Effect of Hydrogen Ion Concentration on the Thermal Death Rate of Bacterium coli. 15:341-356, 1928.

53. Myers, R. P. The Germicidal Properties of Alkaline Washing Solutions, with Special Reference to the Influence of Hydroxyl-ion Concentration, Buffer Index, and Osmotic Pressure. J. Agr. Res. 38:21-563, 1929.

54. Nesbitt, J. B. Phosphorus Removal - the State of the Art. J. Water Pollut. Contr. Fed. 41:701-713, 1969.

55. Ostrum, T. R., C. R. West, and J. J. Shafer. Investigation of Sewage Sump on the Greeland Icecap. J. Water Pollut. Contr. Fed. 34:56-62, 1962.

56. Owen, R. Removal of Phosphorus from Sewage Plant Effluent with Lime. Sewage Works and Ind. Wastes. 25:548-556, 1953.

57. Pelczar, M. J. Jr., and R. D. Reid. Microbiology, 2nd ed. New York, McGraw-Hill Book Co., Inc., 1965. 322 p.

58. Riehl, M. L. Water Supply and Treatment. The National Lime Ass. Washington, D.C. Bulletin 21. 1970.

59. Riehl, M. L., H. O. Hartung, and F. S. Taylor. Effects of Lime Softening. J. Amer. Water Works Ass. 46:493-498, 1954.

60. Rudolfs, W., and H. W. Gehm. Chemical Sewage Coagulation. Part I. Effect of pH. Sewage Works J. 8:195-204, 1936.

61. Rudolfs, W., and H. W. Gehm. Chemical Sewage Coagulation. Part II. Relation between Turbidity, Suspended Solids and BOD. Sewage Works J. 8:204-209, 1936.

62. Rudolfs, W., and H. W. Gehm. Chemical Sewage Coagulation. Part III. Effects of Presettling. Sewage Works J. 8:422-429, 1936.

63. Rudolfs, W., and H. W. Gehm. Chemical Sewage Coagulation. Part IV. BOD of Soluble and Dispersed Materials. Sewage Works J. 8:537-546, 1936.

64. Sawyer, C. N. Some New Aspects of Phosphates in Relation to Lake Fertilization. Sewage and Ind. Wastes. 24:6, 768, 1952. Cited in: Nesbitt, J. B. Phosphorus Removal - the State of the Art. J. Water Pollut. Contr. Fed. 41:701-713, 1969.

65. Schaffer, J. M., and F. W. Tilley. Germicidal Efficiency of Soaps and Mixtures of Soaps with Sodium Hydroxide or with Phenols. J. Agri. Res. 41:737-747, 1930.

66. Schmid, L. A., and R. E. McKinney. Phosphate Removal by a Lime-biological Treatment Scheme. J. Water Pollut. Contr. Fed. 41:1259-1276, 1969.

67. Schuyler, R. Bacteria versus pH in Sugar Beet Waste. M.S. Thesis. Colo. State Univ. Fort Collins, Colo. 1969. 58 p.

68. Scott, R. D., and G. M. McClure. The Hydrogen Ion Concentration of Lime Treated Water and its Effect on Bacteria of the Colon-typhoid Group. J. Amer. Water Works Ass. 11:598-604, 1924.

69. Shaughnessy, H. J., and I. S. Falk. The Effects of Some Electrolytes on the Buffering Capacity of Bacterium coli. J. Bacteriol. 9:559-579, 1924.

70. Shaughnessy, H. J., and C. E. A. Winslow. The Diffusion Products of Bacterial Cells as Influenced by the Presence of Various Electrolytes. J. Bacteriol. 14:69-99, 1927.

71. Stauffer, D. McN. Sewage Purification in America. Eng. News. 28:32-611, 1892.

72. Stauffer, D. McN. Sewage Treatment and Sludge Disposal. Eng. News. 29:198-199, 1893.

73. Streeter, H. W. The Bactericidal Efficiency of the Excess-lime Method of Water Purification. Pub. Works J. 64:17-20, 1933.

74. Thayer, S. E., and O. J. Sproul. Virus Inactivation in Water-softening Precipitation Processes. J. Amer. Water Works Ass. 58:1063-1074, 1966.

75. Thimann, K. V. The Life of Bacteria, 2nd ed. New York, The Macmillan Co., 1963. 909 p.

76. Tilley, F. W., and J. M. Schaffer. Germicidal Efficiency of Sodium Hydroxide, Sodium Carbonate and Trisodium Phosphate. J. Agr. Res. 42:93-106, 1931.

77. Van Arnum, W. I. Use of Lime as a Water Purification Agent at Youngstown. Ohio Conf. Water Purif., 10th Annu. Rep. 1930.

78. Vuuren, L. R. J. van, C. J. Stander, M. R. Henzen, P. G. R. Meiring, and S. H. V. van Blerk. Advanced Purification of Sewage Works Effluent using a Combined System of Lime Softening and Flotation. Water Res. 1:463-474, 1967.

79. Watkins, J. H., and C. E. A. Winslow. Factors Determining the Rate of Mortality of Bacteria Exposed to Alkalinity and Heat. J. Bacteriol. 24:243-265, 1932.

80. Wattie, E., and C. W. Chambers. Relative Resistance of Coliform Organisms and Certain Enteric Pathogens to Excess-lime Treatment. J. Amer. Water Works Ass. 35:709-720, 1943.

81. Winslow, C. E. A., and I. S. Falk. Studies on Salt Action VIII. The Influence of Calcium and Sodium Salts at Various Hydrogen Ion Concentrations upon the Viability of Bacterium coli. J. Bacteriol. 8:215-236, 1923.

U.S. GOVERNMENT PRINTING OFFICE: 1973 546-312/127 1-3